Review

Macula densa neuronal nitric oxide synthase

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Abstract

In the juxtaglomerular apparatus of the kidney the distal tubule returns to its own glomerulus where specialised cells, the macula densa (MD) cells, are located so that they come in contact with the tubular fluid. It is well documented that MD cells sense the distal tubular flow-rate and that when the flow is too high they are activated, which leads to vasoconstriction of the afferent arteriolar, the tubuloglomerular feedback (TGF). Neuronal nitric oxide synthase (nNOS) is largely expressed in the MD cells. Acute inhibition of MD nNOS leads to an enhanced TGF response, indicating that nitric oxide (NO) is an important short-term modulator for volume regulation in the body. By contrast, the NO from the nNOS of the MD cells does not seem to play a role in the long-term adaptations of renin release in low- or high-salt diets.

Keywords: Blood pressure; Hypertension; Nitric oxide; Renal function; Renin angiotensin system

1. Introduction

Nitric oxide (NO) has a wide range of effects throughout the body and has been shown to be involved in vasodilation, cell growth, inflammation, neurotransmission, hormone release and renal sodium handling. Chronic blockade of NO synthesis has been shown to lead to hypertension and tissue damage [1]. Nitric oxide is formed together with L-citrulline from the substrate L-arginine (L-Arg) and oxygen through enzyme nitric oxide synthase (NOS). There are three known isoforms of NOS, the neuronal isoform (nNOS or NOS I), the inducible isoform (iNOS or NOS II) and the endothelial isoform (eNOS or NOS III). The nNOS and the eNOS are constitutively expressed and Ca\(^{2+}\)-dependent, while the iNOS is inducible and not Ca\(^{2+}\)-dependent. However, it has been reported that in the kidney the iNOS is also constitutively expressed [2].

The juxtaglomerular apparatus of the kidney is important in regulating the extracellular fluid volume in the body. It consists of the macula densa (MD) cells, afferent and efferent arterioles, and the mesangium. In this app-

2. Neuronal nitric oxide synthase in the kidney

In the kidney, nNOS is largely expressed in the MD cells [4–6]. The enzyme nNOS has also been found to be expressed in the medullary thick ascending limb [7], the inner medullary collecting duct (IMCD) [8–11] and the principal cells of the cortical collecting duct [12,13]. Neuronal nitric oxide synthase activity is regulated by Ca\(^{2+}\)/calmodulin binding, phosphorylation, and feedback...
inhibition of NO [14]. Another possible mechanism of regulating nNOS in the kidney has been suggested by Rocznia et al. [15] who found that the endogenous nNOS inhibitor, a protein inhibitor of nNOS (PIN), previously isolated from rat hippocampus [16], is also present in the kidney [15]. The PIN was observed to be expressed in the endothelial cells of the glomeruli and the afferent arterioles and vasa recta as well as on the apical surface of IMCD cells in the medulla [15]. Rocznia et al. have previously shown that in the rat remnant kidney after 5/6 nephrectomy, nNOS is downregulated [10]. They suggest that this downregulation is, at least in part, due to an increased PIN expression. The exact degree to which this mechanism operates in the juxtaglomerular apparatus is as yet unclear.

3. Macula densa neuronal nitric oxide synthase and tubuloglomerular feedback

To understand how the TGF mechanism operates it is necessary to define its two different functions, namely its basic function and that of resetting the TGF. The basic function of the TGF is to sense the NaCl load at the MD site, and translate this into a signal that modulates the afferent arteriole tone, keeping the fluid load to the distal tubuli fairly constant. An increased fluid load at the MD site is caused by an increase in the glomerular filtration rate (GFR) due to several mechanisms, increased glomerular capillary pressure, decreased oncocytic pressure, increased $K_e$, etc and impaired tubular reabsorption. The TGF responds by adjusting the arteriolar tone, and thereby the GFR, to counter the increased fluid load.

In early and also in later studies, it was clear that there is an important function in resetting the TGF system. This means that the information transmitted by local factors such as e.g. interstitial pressure, prostaglandins and kinins will determine the specific filtration rate the existing condition will allow. For instance, in dehydration the TGF system is activated to a large extent even though the filtered load is lower than normal to avoid further fluid losses [17]. In the resetting process many different hormones and local factors are involved, angiotensin II (Ang II), prostaglandins, kinin and thromboxane, to name a few [18].

These two different functions of TGF are closely interrelated and splitting the operation of TGF in two is done purely to help understand the process by viewing the resetting process as a second feedback loop modifying the response of the first. Recently there has been substantial progress in understanding the workings of both of these processes.

Today there is little doubt that the initial step in TGF is the sensing of the NaCl load/concentration at the MD site. An increased load will lead to an increased NaCl concentration at the MD site owing to the prevailing electrolyte transport characteristics of the nephron. This sensing step involves the transport of NaCl via a Na, K and 2Cl co-transport mechanism [19–21]. The next step, the signalling between the MD cells and the afferent arteriole, has been more difficult to elucidate. Intracellular calcium has been regarded as one possible link. However, the calcium changes following an increased NaCl load past the MD cells are small and conflicting results have been reported by different groups of investigators [22,23]. For the time being it is not clear to what extent calcium concentration changes are involved in the signalling. What happens next in the signalling process is not yet clear either; however, this and another group of investigators have found that adenosine A$_1$ receptor (A$_1$R) knockout mice lack the TGF response [24,25]. This finding strongly suggests that the release of adenosine is an essential step in TGF signalling at some point. The mechanism for adenosine release is not known, but as suggested by Osswald et al. [26], it may depend on the breakdown of adenosine triphosphate (ATP) in response to increased transport of electrolytes through the MD cells, which would lead to the formation of adenosine at an elevated rate.

4. Resetting of tubuloglomerular feedback sensitivity

In exploring the second feedback loop which gives rise to a resetting of TGF sensitivity, as described above, there has been considerable progress concerning its function. A number of different hormones and local factors are important for setting the level of sensitivity of the TGF. Recently, it has been made clear that NO produced from the nNOS located in the MD cells is important for the setting of the sensitivity of the TGF system. Acute blockade of the nNOS in the MD cells increases the TGF sensitivity to a large extent without affecting the arterial blood pressure (Fig. 1) [27–30]. These results indicate that NO production plays an important role in the determination of TGF sensitivity in the control situation. Recently, in a study by Ren et al. [31] it was suggested that the inhibitory effect of MD-derived NO on the TGF is via the stimulation of soluble guanylate cyclase. This in turn generates cyclic guanosine monophosphate (cGMP) and activates cGMP-dependent protein kinase. Ren et al. postulate that MD-derived NO acts directly on the MD cells rather than diffusing to the afferent arteriole and exerting its effect there [31].

Many investigators have studied the effect of different salt diets on MD nNOS expression. In the kidney, cortical and medullar nNOS is stimulated in different situations (Table 1). The medullar nNOS has been shown to increase after high sodium intake [32]. The duration of high-salt treatment may have an influence. Rocznia et al. [9] found that after 3 days of a high-salt diet the nNOS expression in the cortex was decreased, while in the IMCD nNOS protein expression had increased. After 3 weeks of a high-salt diet the increase in IMCD nNOS was no longer
Fig. 1. Tubuloglomerular feedback (TGF) response measured in vivo using Stop-flow pressure micropuncture technique in control rats and rats treated acutely [7-nitro indazole a (7-NI a)] for 1 week (7-NI 1w) and 4 weeks (7-NI 4w) with the nNOS inhibitor 7-NI. Bars represent the percentage change in proximal tubular stop–flow pressure (ΔP_{SF}) between 0 (no TGF stimulation) and 40 nl/min (full stimulation) of loop of Henle perfusion and demonstrate the reactivity of the TGF. The graph demonstrates that NO produced from the nNOS located in the MD cells is important for the setting of the reactivity of the TGF system. These results indicate that NO production plays an important role in the determination of the TGF sensitivity in the control situation.* , P<0.05 vs. ΔP_{SF} in control rats. Reproduced from [27].

Macula densa nNOS, however, decreased after a high-salt diet [33]. Low-salt diets have been reported to increase the nNOS expression in MD cells [33,34]. The increased nNOS immunostaining after a low-salt diet seems to be independent of Ang II or AT_1 receptors [34]. Altered plasma Ang II levels have been reported to also cause parallel changes in renal nNOS expression, which suggests that Ang II stimulates nNOS expression [35–37]. Supporting this is a study by Nadaud et al. [38], who found that a combination of deoxycorticosterone acetate (DOCA)-salt loading and hypertension decreases nNOS mRNA levels. Even if nNOS expression is increased after low-salt treatment the NO produced from MD nNOS is not elevated [39,40]. This is probably due to limited delivery and/or uptake of L-Arg in this situation [39,41]. The availability of L-Arg to the MD cells may also be an important limiting factor for the release of NO in normal salt-treated rats [28].

Aminoguanidine, the effects of which are fairly unspecific but which predominantly inhibits iNOS, had no influence on the TGF sensitivity, indicating that iNOS does not play an important role in the resetting of the TGF sensitivity [28]. By contrast, the administration of 7-nitro indazole (7-NI), a nNOS inhibitor, into the tubular fluid has been reported to evoke a near-maximal response in comparison with inhibition by Nω-nitro-L-arginine (L-NNA). 7-Nitroindazole is a relatively selective inhibitor of nNOS [42] and should therefore exert its major renal inhibitory effects on nNOS in the MD cells. It consequently seems reasonable to assume that in the setting of the sensitivity of the TGF mechanism the nNOS is the isoform of greatest importance, at least in the acute situation. The TGF response is increased if the nNOS is inhibited acutely but in the long run can reset back to the normal level (Fig. 1) [27,43]. We found the TGF response in rats treated with 7-NI for 4 weeks to be normal [27] and other investigators have found that nNOS knockout mice have a normal TGF response [43]. Consequently, a long nNOS inhibition or deficiency is probably associated with compensatory adaptations.

Long-term inhibition of nNOS in rats has been shown to increase blood pressure (Fig. 2) [27]. In acute experiments 7-NI has been shown to inhibit nNOS without affecting blood pressure [28,30], while still being almost as potent as unspecific L-arg analogues in enhancing TGF responsiveness and reducing GFR [28]. We found that rats treated

Table 1

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<th>Treatment</th>
<th>Low-salt</th>
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<tr>
<td>Cortex nNOS↑ [33,34]</td>
<td>nNOS↓ [9,33]</td>
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<td>Medulla nNOS→ [33]</td>
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also recently found that the administration of Ang II to the isolated perfused ascending limbs of Henle with attached glomerulus increases MD cell calcium by as much as 100 nM. As can be expected from a constitutive NOS, we found that an increase in calcium concentration gives rise to an increase in NO production [46].

An intriguing question, since Ang II gives rise to a large increase in NO production, is how this hormone sensitises the TGF. In speculation about the mechanism behind this sensitisation the following theories could be advanced: obviously, if we accept the recent finding of the importance of adenosine as a mediator of the TGF, elevated adenosine levels or an adenosine effect would increase the TGF sensitivity while increased NO production would decrease the sensitivity. When the Ang II level is increased it leads to an increase in the calcium concentration in the MD cells (Fig. 3). This increased calcium concentration with calmodulin could give rise to an increased production of NO. At concentrations around the physiological level, NO is able to inhibit oxygen metabolism in kidney tubular cells [47]. A decrease in oxygen metabolism is known to reduce the amount of ATP, which in turn will decrease electrolyte transport rates and also increase adenosine production [48]. In a study by Beach et al. [49], adenosine release in a kidney cell suspension was stimulated by hypoxia. The resultant increase in adenosine was completely blocked by furosemide or ouabain, which suggests that this adenosine release was related to ion transport and oxidative metabolism [49].

Another mechanism that may be important in the sensitisation of the TGF with increased Ang II levels is the potentiating effect that Ang II has on adenosine [50]. The sensitisation process of the TGF may, then, depend on an increased vasoconstriction by adenosine, which in turn is dependent on the presence of a large concentration of Ang II that will potentiate the contractile response. It is interesting to note that Pflueger et al. [51] found a tenfold increase in vascular response to adenosine when NO production was blocked.

If sensitising effects are defined as effects which increase adenosine effect or concentration, and if desensitising effects are viewed as effects that increase the production rate of NO, the sensitivity of the TGF system will depend on a balance between these two important factors.

5. Sensitisation–desensitisation of the tubuloglomerular feedback

In an attempt to study the NO release from the MD cells and its mechanisms we studied isolated perfused ascending limbs of Henle with attached glomerulus by confocal microscopy [45]. The findings indicated that an increased distal delivery of fluid to the MD site, simulated by an increased NaCl concentration at the MD site, releases NO while a decrease in NaCl concentration did not result in any release of NO [45]. These results indicate that as soon as the flow is increased there is a release of NO. We have

6. Macula densa neuronal nitric oxide synthase and renin

Restrictions in sodium intake are associated with markedly elevated plasma renin concentrations (PRCs); a high-salt diet, by contrast, is associated with a lowered PRC [52–54]. Nitric oxide, generated by nNOS in the MD, may be central to this response [55]. The role of NO in renin release is still controversial. Many investigators have reported that NO stimulates renin secretion, both in vivo
Fig. 3. Diagram representing a possible explanation of angiotensin II (Ang II)’s involvement in sensitising the tubuloglomerular feedback (TGF).

In contrast to our findings, Beierwaltes [55] found that the effect of 7-NI on plasma renin and renal renin content after 5-day treatment with 7-NI and a normal or low-salt diet decreased the low salt-induced elevations in plasma renin activity. We have no explanation for the divergence in results. However, in the study by Beierwaltes the renal renin content was equal in all groups [55]. This is striking, because adaptation of the renin–angiotensin system in the long term usually takes place by changing the number of renin-producing cells [66].

In the study by Zanchi et al. [67] the PRC increased after NOS inhibition in rats receiving a normal salt diet. In
a group receiving a low-salt diet, the PRC was also increased, but t-NAME had no further effect. The inability of NOS blockade to modify the long-term renin response to a low-salt intake is in line with the results in our study [65] and is further supported by studies with nNOS knockout mice [68,69]. These mice show a normal response in renin release following low salt intake [68]. Another study [69] showed that neither eNOS nor nNOS is essential for up- or downregulation of renin expression in the eNOS and nNOS-deficient mice. Despite these findings the renal renin mRNA expression, during a control situation, in the eNOS knockout mice was only half of that found in the wild-type mice. However, in the nNOS knockout mice in contrast to the wild-type mice renin mRNA expression was unaltered [69].

In the long run NO from MD nNOS is probably not important for the up- or downregulation of renin secretion even though, in the acute state of nNOS inhibition, it seems to be important for the increase in renin release following a low-salt diet.

7. Macula densa cyclo-oxygenase 2 and neuronal nitric oxide synthase

Cyclo-oxygenase 2 (COX-2) is an inducible isofrom of cyclo-oxygenase (COX) and metabolises arachidonic acid. It generates prostaglandins and thromboxanes that are important for the vascular tone of the kidney, GFR and tubular reabsorption mechanism [70]. In the renal cortex, COX-2 is restricted to the MD cells and the cells in the thick ascending limb near the MD [71]. It has been shown that COX-2 increases when renin is high, such as during low-salt treatment, renovascular hypertension or volume depletion [71–75]. It is possible that NO is a mediator for the increased renal cortical COX-2 expression seen after volume depletion [76]. If COX-2, on the other hand, is not present (in COX-2 knockout mice) the renin activity is decreased [77] and if COX-2 is inhibited the expected renin increase after such treatment as low salt is abolished [68,78]. This shows that COX-2 is critically involved in the MD control of renin secretion and possibly is the source of the prostaglandins participating in the signalling pathway between the MD and the renin-producing granular cells. During increased activation of TGF-dependent vasoconstrictor signals [79], COX-2 has been reported to generate vasodilatory metabolites in response to increased NOS activity and in this way to counteract the constrictive response of the afferent arteriole. However, it has not been established whether NOS and COX-2 are independent, even if they are regulated in the same direction by the same stimuli [80].

Short-term NOS inhibition leads to decreased GFR, increased TGF sensitivity and, in combination with a low-salt diet, an increased PRC. After a long-term inhibition of nNOS GFR, the PRC and TGF response return to normal while the blood pressure is elevated.

In summary, MD-derived NO is of great importance in the function of the TGF, both in the basic TGF loop and in the resetting after such events as volume expansion. Renin secretion is not affected by long-term inhibition of NOS or in the nNOS knockout mice, while in the acute phase it appears to be involved in the regulation of renin secretion. Cyclo-oxygenase 2, contained in the MD cells, is critically involved in the MD control of renin secretion.

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References


