CHAPTER V

DISCUSSION AND CONCLUSION

Increasing evidences have suggested a complex interaction between ANG II and NO (Hennington et al., 1998; Olson, 1997: Pueyo, 1998). Moreno et al. (2002) have shown that acute as well as prolonged infusion of ANG II could upregulate NOS levels in renal tissues. On the other hand, administration of NO donor, sodium nitroprasside, induces 4.5 fold increase of basal renin secretion rate in isolated perfused rat kidney (Kurtz et al., 1998). In addition, NO could stimulate renin secretion in both conscious (Persson et al., 1993) and anesthetized animals (Johnson et al., 1994).

Several studies have indicated that ureteral obstruction increases various mediators including ANG II (Frokiaer et al., 1992; Ishidoya et al., 1995) and NO (Pimentel et al., 1993). Besides vasoconstrictive effects that could impair renal hemodynamics, ANG II plays a central role in initiation and progression of tubulointerstitial fibrosis and renal damage in ureteral obstruction (Klahr and Morrissey, 1998; Klahr et al., 1995). ANG II upregulates the expression of TGF-β (Kaneto et al., 1993; Pimentel et al., 1995), TNF- α (Gou et al., 2001), and other growth factors and cytokines (Klahr and Morrissey, 1998; Sharma et al., 1993; Klahr and Morrissey, 1997; Bander et al., 1985; Hammad et al., 2000) that lead to the accumulation of extracellular matrix proteins and, eventually, fibrosis. ACEI as well as ARA are found to blunt the expression of such substances

in concomitant with amelioration of histological changes (Ishidoya et al., 1995; Klahr and Morrissey, 1997; Kaneto et al., 1994).

Several studies have shown that NO protein expression and, thus, plasma as well as urine NO were elevated in UUO (Morrissey et al., 1996). NO could counteract the alteration in renal hemodynamics induced by ANG II (Aki et al. 1997). Apart from vasodilating affect, NO could also affect tubulointerstitial fibrosis (Morrissey et al., 1996). NO obtained from eNOS exerts antifibrosis property while iNOS-derived NO enhances fibrosis (Huang et al., 2000). In addition, L-arginine administration in UUO animals could restore not only renal function but also improves nephropathy (Chang et al., 2002; Hegarty et al., 2001; Lanzone et al., 1995; Schulsinger et al., 1997). By contrast, treatment with NOS inhibitor results in sustained renal damage caused by ureteral obstruction (Chevalier et al., 1992). Therefore, during ureteral obstruction, it seems to be that ANG II is stimulated first and then activates NOS later. However, the conclusive information remains unclear.

To date, there are no such data showing the effect of blockade in renin angiotensin system on either renal eNOS or iNOS protein expression during ureteral obstruction. The present study tries to elucidate this aspect. The results show that eNOS protein is well expressed constitutively in cytoplasmic area of renal tubular epithelial cells and glomerulus. Such findings concur with previous studies showing that eNOS expression is located in glomeruli, preglomerular vasculature, proximal tubules, thick ascending limbs, and collecting ducts in normal rats (Terada et al., 1992; Ujiie et al., 1994). One day and seven days after UUO, the present data show that the renal eNOS protein expression increased in both the cortical

and medullary region (Figure 4). The explanation for this alteration may involve some mechanisms as follow.

It has been shown that UUO could enhance NOS activity in microdissected segments of IMCD (Valles and Manucha, 2000), and could increase eNOS protein expression in rat kidney (Hegarty et al., 2001). Moreover, UUO also upregulates eNOS mRNA level in renal tissue biopsied from obstructed kidneys of 18 children at the time of pyeloplasty (Valles et al., 2003). As noted, the higher level of ANG II during UUO is one of the main mechanisms activating Ca^{+}-dependent NOS activity and protein expression (Moreno et al., 2002; Hegarty et al., 2001). The induction of NOS expression and, thus, NO production in UUO could counteract the vasoconstrictive effect of ANG II which reduces renal blood flow (Moody et al., 1975; Solez et al., 1976).

Ureteral obstruction initiates renal ischemia (Klahr 2001; Moody et al., 1975). In obstructed kidney, it has been found that ipsilateral renal vascular resistance is increased whereas renal blood flow is declined (Moody et al., 1975). Furthermore, the radioactive albumin accumulation technique has revealed a progressive reduction in inner medullary plasma flow within a few hours after UUO in rat (Solez et al., 1976). Under normal physiological conditions, in the rat kidney, the tissue partial oxygen pressure is 50 mmHg in cortex and is 10-20 mmHg in medulla (Brezis et al., 1991). During ureteral obstruction, the reduction of oxygen consumption is observed in renal tissue (Kurokawa, 1982).

The eNOS protein expression is regulated by hypoxia. The cell culture studies showed that the amount of eNOS mRNA and protein are increased in porcine coronary arteriolar endothelial cells (Xu et al., 1995)

and in bovine aortic endothelial cell (Arnet et al., 1996). Moreover, the rats subjected to hypoxia (9 - 10% oxygen exposure) has the increment of both eNOS RNA and protein expression in various tissues including kidney (LeCras et al., 1996; Gess et al., 1997). Therefore, the induction of eNOS expression in the present study may result from decrement of renal tissue perfusion. In addition, a previous study has demonstrated that ureteral obstruction in mice causes proximal tubule hypoxia and necrotic injury (Cachat et al., 2003).

During ureteral obstruction, while the renal blood flow is gradually declining, the ureteral pressure is progressively greater (Lanzone et al., 1995; Schulsinger et al., 1997; Solez et al., 1976; Wrigh and Briggs, 1979). This results in increased wall tension, renal tissue stresses, and then renal atrophy as well as necrosis (Border and Noble, 1998; Sigmon and Beierwaltes, 1993; Wrigh and Briggs, 1979). This may be explained by the increased oxidant stress, together with downregulation of antioxidant mRNA expression in the rat renal cortex post-UUO (Ricardo et al., 1997). In addition, the studies in bovine aortic endothelial cells show that the cells exposed to mechanical stretch induces eNOS expression (Awolesi et al., 1995; Ziegler et al., 1998). Thus, the increased renal eNOS expression in the present study may, in part, be activated via the mechanical stretch pathway.

In the present study, both ACEI and ARA slightly decreased renal eNOS protein expression in 1-day UUO rats. This may be caused by the inhibition of renin angiotensin system and, then, less activation of NOS. Of interest, the eNOS protein expression was progressively increased in 7-day UUO group although the animals still received either ACEI or ARA. The mechanisms for this alteration are more complex.

The two distinct subtypes of ANG II receptor have been defined and designated as type 1 (AT_1) and type 2 (AT_2) receptors (Arima and Ito, 2001). Most of the well-characterized actions of ANG II are now generally considered to result from stimulation of AT_1 receptor, whereas AT_2 receptor may exert opposite effects against AT_1 receptor (Arima and Ito, 2001.

Regard to biological effect of AT_2 activation, a recent study has demonstrated the antifibrotic phenomena induced during obstructive nephropathy (Morrissey and Klahr, 1999). Moreover, accelerated renal interstitial fibrosis and collagen depositions have been observed in adult AT_2 receptor null mutant mice during unilateral ureteral obstruction (Ma et al., 1998). In addition, the AT_2 receptor has also been found to blunt proliferation of vascular smooth muscle cells (Yamada et al., 1996), coronary endothelial cells (Stoll et al., 1995), and renomedullary interstitial cells (Maric et al., 1998).

In 7-day ARA-treated animals, the present study shows that the eNOS protein expression was sustained in the cortex and was progressively increased in the medulla. This may be the result of the more bindings of ANG II to AT_2 receptor and then activate nitric oxide pathway. It has been reported that AT_2 receptor mediates the production of nitric oxide in renal interstitial cells (Cachat et al., 2003; Siragy and Carey, 1997).

The enhancement of eNOS protein expression in a long-term ACEI treatment also was observed in the present study. As well known that ACEI not only increases bradykinin (BK) concentrations but also activates bradykinin B_1 receptor (Ignjatovic et al., 2002). After activation of B_1 receptor, elevated intracellular calcium is observed and then NO release

is noted in cultured cells (Ignjatovic et al., 2002). In addition, the more available of bradykinin during ACEI treatment could bind to the B_2 receptor as well, the binding of which has been shown to decrease renal fibrosis induced by ureteral obstruction (Schanstra et al., 2002).

It has been shown in the last few years that ANG II can stimulate the synthesis and release of endothelin-1 (ET-1) in endothelial cells or vascular smooth muscle cells (Ferri et al., 1999; Rajagopalan et al., 1997). In ureteral obstruction, the expression of renal endothelin converting enzyme-1 and endothelin-A-receptor (ET_A) mRNA in rats are enhanced (Feldman et al., 2000). Apart from activation of bradykinnin pathway, ACEI has an additional effect on endothelin and its receptor expression (Moridaira et al., 2003). Administration of ACEI could ameliorate the increased expression of ET-1 mRNA in the obstructed kidney but no changes in ET_A mRNA expression have been observed. Interestingly, ACEI markedly increased endothelin-B (ET_B) mRNA and protein expression in the obstructed kidney (Moridara et al., 2003). In endothelial cell culture, Hirata et al (1992) have shown that ET_B receptor agonist stimulates the production of NO and cGMP and then relaxes vascular smooth muscle (Karaki et al., 1997). Therefore, the increased eNOS protein expression during ACEI treatment in the present study may mediate via activation of ET_B receptor. However, a further study in this regard is needed.

Regarding iNOS expression, it has long been established that iNOS is typically induced by cytokines and lipopolysaccharides (Wolf et al., 1997). Many studies have demonstrated iNOS expression in renal epithelial cells, such as proximal tubular and medullary thick ascending limb of

Henle's loop (Markewitz et al., 1993; Kone et al., 1995). In ureteral obstruction, the results related to iNOS expression have yielded controversial data. The in vivo study by Fitzgerald et al. (2001) has found that UUO enhances iNOS mRNA and protein expression in medulla whereas the expression is diminished in cortex. Recently, Miyajima et al. (2001) have shown that the iNOS mRNA level in the obstructed kidney is peaked at day 3 and decreased significantly by day 7 but the level is less in day 14. By contrast, the renal tubular cells subjected to mechanical strain has an increased iNOS expression but no increase in NO production (Hegaety et al., 2002). The regulations of iNOS expression are more subtle.

The upregulation in iNOS is mainly mediated via ANG II activated cytokine-stimulated NO synthesis by increase in magnitude of iNOS mRNA accumulation (Nakayama et al., 1994; Ujiie et al., 1994). On the other hand, transforming growth factor β (TGF-β) induced by ANG II (Pimentel et al., 1995; Kihara et al; 1999) and mechanical stretch (Miyajima et al., 2000) reduces iNOS expression as well as NO production (Miyajima et al., 2000; Klahr and Morrissey, 2002). TGF-β could suppress iNOS expression through various pathways. In macrophages, TGF-β reduces the stability and rate of degradation of iNOS protein (Vodovotz et al., 1993). Moreover, in smooth muscle cells, TGF-β reduces the expression of iNOS at the transcriptional (Perella et al., 1994) and translation (Finder et al., 1995) levels. Furthermore, TGF-β also decreases the numerous transcriptional factors that induce transcription of the iNOS gene. These factors are NF-kB (Xie et al., 1994), interferon regulatory factor-1 (IRF-1) (kamijo et al., 1994), and AP- 1 (Chung et al., 1996).

Besides the potent effect on iNOS expression, TGF-β could reduce the activity of iNOS without affecting its expression. TGF-β can enhance the activity of arginase in macrophages, thereby, limiting the availability of L-arginine, the substrate for all isoforms of NOS (Boutard et al., 1995). In addition, TGF-β can suppress the production of tetrahydrobiopterin (Schoedon et al., 1993), an obligatory cofactor for NO synthesis. However, the present study demonstrated no changes in iNOS protein expression data. These data agree with the study by Knerr et al. (2001). They showed that iNOS mRNA levels as comparable quantities in patients with congenital ureteropelvic junction obstruction compared with controls. The exact explanations for this phenomenon are unclear. It may be due to the neutralizing processes between stimulation and inhibition pathways on iNOS expression. Further studies in more details are required.

Collectively, the ANG II-NO interactions are more subtle and complex. The counterbalancing effects of ANG II and NO could regulate not only renal function but also renal tissue integrity. The modulations with each other also are mediated via multiple cascades to exert system homeostasis.

In conclusion, the present data are the evidence of UUO model in that angiotensin blockade could attenuate renal eNOS protein expression in 1-day UUO but not in 7-day UUO. The inhibition of angiotensin system ameliorates increased NO production and nephropathy induced by UUO.