Renal effects of opioid exposure: Considerations for therapeutic use

Kalpna Gupta, PhD
Marc L. Weber, MD

ABSTRACT

In recent years, the discovery of peripheral opioid receptors has challenged the dogma of opioids interacting exclusively with the central nervous system. In this article, we describe the current understanding of the roles of opioids and opioid receptors in renal physiology and pathophysiology. The renal response to opioid exposure varies depending upon the specific opioid agonist, dose, and duration of exposure. The known acute effects of opioids on the kidney impact salt and water balance. The chronic effects of opioid exposure on kidney function are largely unknown, but collapsing glomerulopathy has been associated with chronic heroin abuse. Opioid exposure can lead to both physiological and architectural renal changes, and this may have important clinical implications. Since opioids are often used for pain management in patients with existing kidney disease, their role in kidney function warrants attention.

Key words: opioid, morphine, kidney, renal function, endothelium

INTRODUCTION

Opioids have been used for nearly six centuries for pain control, including by Hippocrates (460-377 BC), considered the founding father of medicine.[1] The clinical use of opioids for pain control has exploded as more opioid agonists have become available. However, the clinical significance of opioid use goes well beyond pain control and requires an understanding of the drugs' role in organ disease, especially when treating patients with pre-existing conditions. Opioid actions, previously believed to be confined to the central nervous system, have attracted considerable attention for their existence and possible functions in peripheral systems.[2-4] Increased understanding of opioid signaling pathways has opened the door to new vistas in terms of understanding their role in growth, survival, and vital physiological functions such as vasodilation.[5] Our laboratory, at the University of Minnesota, showed that morphine stimulates angiogenesis-dependent tumor growth and ischemic wound healing.[6,7] We also found evidence that opioids and opioid receptors play an important role in the maintenance of normal kidney physiology in mice.[8] Data are beginning to emerge that suggest both exogenous and endogenous opioids have important actions on the kidney. The renal response to opioid exposure varies depending upon the specific opioid agonist, dose, and duration of exposure (acute vs. chronic). The text to follow explicates the renal effects of endogenous and exogenous opioids, their receptors, and the potential renal consequences of acute and chronic opioid exposure.

OPIOID RECEPTORS

Opioid receptors (ORs), once thought to be expressed exclusively in the central nervous system, have also been identified in the kidney, and acute opioid administration has been shown to have various renal effects.[3,4] To date, four different ORs have been cloned: μ, δ, κ (MOR, DOR, and KOR, respectively), and nociceptin (ORL1).[9,10] Like other receptors, opioid receptors have specific agonists and antagonists.

Agonist selectivity is thought to be attributed to the first and third extracellular loops of MOR, the second extracellular loop of KOR, and the third extracellular loop of DOR. Opioid receptors have about 60 percent identity, with the highest homology in the transmembrane region and the most diversity in the N and C termini.[11] DOR was first characterized in the mouse vas deferens. There are several isoforms of OR, with various affinities for opioid ligands. KOR has a high affinity for dynorphin A. MOR has a high affinity for morphine, although morphine interacts with all ORs. ORL1 has been identified in humans, rats, and mice, with over 90 percent conserved homology between species.[9] ORL1 is included in the OR family based on structural characteristics, in spite of little pharmacologic homology. Opioid receptor expression can be modulated by proinflammatory cytokines and growth factors.[11] For example, interleukins (IL) 1 and 6 and vascular endothelial growth factor (VEGF) stimulate MOR expression, while nerve growth factor and IL4 stimulate DOR expression in different cell types.[12] Thus, the

changing microenvironment in different pathological conditions may have an effect on opioid receptor activity, depending upon the receptors' expression.

SIGNALING

Opioid receptors belong to a superfamily of seven transmembrane G-protein-coupled receptors (GPCRs). Upon activation, opioid receptors are coupled to Gi/Go proteins, which interact with several downstream effectors to inhibit adenylate cyclase and voltage-gated Ca^{++} channels.[11] However, chronic activation leads to cyclic adenosine 3',5'-monophosphate (cAMP) superactivation and increased cAMP.[9] Because cAMP is a survival factor for endothelial cells, acute and chronic activation can be a matter of death and survival, respectively, in endothelium. Endothelium and vasculature play critical roles in kidney pathology and function. Therefore, in this context, it is reasonable to believe that short- and long-term effects of opioid exposure on the kidney can be opposite each other.

ORs (similar to other GPCRs) are capable of signaling via the family of mitogen-activated protein kinases (MAPKs). MAPKs constitute a family of seronine/threonine kinases that are important in cell processes such as growth, response to external stimuli, and apoptosis.[13] Three major subfamilies of MAPK exist, including the p44/p42 (ERK1/ERK2), JNK, and p38. The p44/p42 pathway is activated by growth factors, while the JNK and p38 MAPK pathways are also stimulated by external stressors such as inflammation. It is theorized, and shown in vitro, that activation of MAPK allows GPCR agonists to modulate such diverse molecular events as cell proliferation, differentiation, and survival.[14] MOR, DOR, and KOR have the ability to signal through MAPKs in various cell types.[15-17] MOR, DOR, and KOR activation in endothelial cells results in stimulation of the p44/p42 MAPK pathway and subsequent proliferation.[6]

Figure 1 shows that ORs have the ability to signal through cAMP and PI3 kinase in addition to MAPK.[18-20] ORs stimulate vasodilatory, cytoprotective, and growth-promoting signaling by activating nitric oxide, hemoxygenase-1, cycloxygenase-2, and signal transducer and activator of transcription. Classical activation of these pathways involves growth factor stimulation of a receptor tyrosine kinase (RTK), which ultimately leads to downstream signaling and p44/p42 MAPK activation.[21] However, transactivation of RTK by GPCR has been well described.[22,23] This is also true for opioid receptors. For example, MOR transactivates epidermal growth factor receptor[24] and VEGF receptor 2/Flk-1.[25] DOR and KOR have also been indirectly associated with RTK transactivation.[15] Stimulation of vasodilatory, cytoprotective, and growth-promoting mechanisms by ORs may be critical in kidney function (Figure 1).

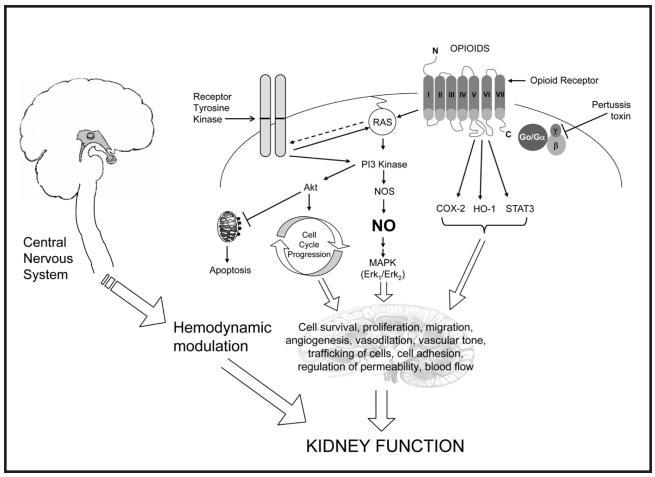

Figure 1. Proposed effect of opioids on kidney function. NOS, nitric oxide synthase; NO, nitric oxide; MAPK, mitogen-activated protein kinase; COX-2, cyclooxygenase-2; HO-1, hemoxygenase-1; STAT3, signal transducer and activator of transcription.

RENAL EFFECTS OF ENDOGENOUS OPIOIDS

Endogenous opioids have a profound effect on kidney homeostasis of salt and water balances (Table 1). Surgically manipulated, stressed rats have an antinatriuretic response to administration of the endogenous opioid dermorphin, without changes in renal blood flow, glomerular filtration rate (GFR), or blood pressure; this response was blocked by naloxone.[26] In a different study, low-frequency renal nerve stimulation led to an antinatriuretic response that was also inhibited by naloxone.[27] When viewed together, these studies outline the importance of stress-induced activation of the peripheral endogenous opioid system on kidney function.

Considerable evidence supports the participation of endogenous opioids in renal function.[2,28-31] Under basal conditions, the opioid system remains quiescent, but when dietary sodium is restricted, central opioid pathways are activated as a mechanism to retain a maximum of sodium.[30,32,33] Other data suggest that opioids modulate renal function via central and sympathetic nervous system dependent and independent pathways.[34] For example, intracerebroventricular (ICV) injection of dermorphin produced an increase in urine flow rates in denervated animals as well as in controls.[32] These animals also displayed decreases in urine sodium excretion, without alterations in GFR or effective renal plasma flow. These alterations were assumed to have been prevented by pretreatment with a selective MOR antagonist, suggesting a direct effect on renal tubular absorption via renal MOR. KOR agonists induce a profound diuretic and antinatriuretic response involving both central and peripheral mechanisms which are yet to be defined.[30,33,35-37] Beyond their physiological effects,

Table 1. Role of opioid receptors in renal function				
	MOR agonist	**DOR agonist**	**KOR agonist**	**ORL1 agonist**
Renal physiologic effects	Antidiuretic (H)[43]	Aquaresis Natriuresis[46]	Aquaresis Antinatriuresis[30,33,35-37,45]	Aquaresis[47]
Kidney cell effects	Proliferation of: – interstitial cells[50] – mesangial cells[8,51] – epithelial cells[49]	Undefined	Proliferation of mesangial cells[8]	Undefined
CNS dependence	+/-	+	+/-	+/-
Therapeutic uses	Uremic pruritis (naltrexone as an antagonist)[57]		Diuretic (Nirvoline)[58]	

endogenous opioids may promote pathological structural changes within the kidney. β-endorphin amplifies the proliferative effect of IL1 on cultured mesangial cells.[38] Given this data, it is interesting to consider whether endogenous opioids play a pathologic role in the progression of chronic kidney disease. Indeed, significantly elevated plasma β-endorphin levels were found in patients with uremia and chronic renal failure and in patients on dialysis.[39-41]

RENAL EFFECTS OF EXOGENOUS OPIOIDS

Acute effects

Morphine induces a transient, dose-dependent reduction in blood pressure and a dose-dependent elevation in atrial natriuretic peptide (ANP) in both control and denervated animals.[42] The reduction in systemic arterial blood pressure caused by morphine and other MOR agonists can cause a marked reduction in urine output as a result of a secondary decrease in renal hemodynamics and inhibition of baroflex pathways. These lead to an increase in antidiuretic hormone secretion and augmentation of central sympathetic outflow to the kidneys, thus bringing about the diminished output. Renal responses to morphine exposure are dependent upon the integration of several different actions, including ANP release, decreased arterial pressure, subsequent activation of sympathetic nerves, and direct effects on the kidneys.[34]

Morphine, a MOR agonist, is one of the most common substances used in clinical settings. Acute administration of morphine in relatively high doses leads to a decrease in urine output, while lower doses lead to increased urine output and an increase in GFR.[43] The reduction in systemic arterial blood pressure caused by morphine can also decrease GFR. Acute KOR-agonist administration produces a profound diuretic and antinatriuretic response, the mechanism of which is unclear.[44,45] DOR agonists acutely promote diuretic and natriuretic effects.[46] Stimulation of the ORL1 receptor induces a dose-dependent aquaresis by vasopression-independent inhibition of aquaporin 2.[47]

Opioid exposure results in myriad effects outside the realm of antinociception and has been shown to induce a proliferative phenotype in a variety of kidney cell types. Seven days of morphine exposure significantly altered the presence of microprojection on podocytes, as assessed by scanning electron microscopy.[48] Morphine exposure over 48 hours was shown to lead to proliferation of glomerular epithelial cells at low doses and apoptosis at higher doses.[49] Renomedullary interstitial cells underwent proliferation and had increased matrix deposition in response to morphine compared to vehicle.[50]

Chronic effects

The renal consequences of chronic exposure to specific opioid receptor agonists are unknown. The only example of an opioid potentially inducing renal injury is the poorly characterized heroin-induced nephropathy (HIN), which is characterized by collapsing glomerulopathy, a variant type of focal and segmental glomerulosclerosis (FSGS).[1,3,51,52] There is also debate as to whether HIN truly exists or is related to contaminants injected with heroin. The data for or against HIN itself are based largely on case reports and speculation. However, there is a growing literature on the in vitro effects of morphine (a metabolite of heroin) on kidney cells of various types. Morphine exposure induces proliferation of cultured rat mesangial cells, suggesting that

opioids may play a significant role in mesangial expansion.[50] Furthermore, morphine has been shown to increase superoxide production in kidney cells.[49] Oxidative stress is a potential mechanism by which opioids may in some way contribute to the progression of chronic kidney disease. One study showed that rats exposed to long-term intraperitoneal morphine had elevated creatinine values and increased vacuolization in tubular cells compared to controls.[53] It is important to note that classic lesions of FSGS are initiated with mesangial cell hyperplasia and mesangial expansion.[54,55]

THERAPEUTIC CONSIDERATIONS

Peripheral effects of opioids may have clinically beneficial aspects, as well. For example, naltrexone has been used to treat the pruritis associated with chronic kidney disease.[56] Furthermore, opioid receptor agonists can potentially be used as diuretics to treat edematous states associated with cirrhosis or congestive heart failure. Niravoline, a KOR agonist, has been shown in rats to produce a superior aquaresis compared directly to an ADH V2 receptor antagonist.[57]

CONCLUSION

Given the increased utilization of opioids for acute and chronic pain control in a number of disease processes, it is timely to define nonanalgesic renal effects of opioids. Experimental data show that opioids are likely to have physiological renal effects. It remains to be seen whether the observed stimulation of proliferation of kidney cells enacted by endogenous and exogenous opioids translates into a clinically relevant effect. Furthermore, the differential effects of opioid receptors on renal structure and function raise therapeutic implications. Thus, a better understanding of the mechanisms by which opioids exert renal effects is required in order to use them in a more clinically beneficial manner.

In the year 2000, the number of patients with chronic kidney disease (CKD) progressing to a point necessitating renal replacement therapy was over 370,000. This number is projected to double by 2010.[58] This alarming figure represents a public health crisis in terms of morbidity, mortality, and healthcare costs. The possibility that opioids may in any way contribute to the progression or therapy of CKD is a novel idea that requires more attention.

ACKNOWLEDGMENTS

The authors extend their thanks to Ms. Carol Taubert for the preparation of the artwork. This work is supported by NIH Grants HL68802 and CA109582.

Kalpna Gupta, PhD, Division of Hematology, Oncology and Transplantation, University of Minnesota, Minneapolis, Minnesota.
Marc L. Weber, MD, Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, Minnesota.

REFERENCES

1. Booth M: *Opium: A History*. New York: St. Martin's Press, 1998.
2. DiBona GF, Jones SY: Role of endogenous peripheral opioid mechanisms in renal function. *J Am Soc Nephrol*. 1994; 4: 1792-1797.
3. Neidle A, Manigault I, Wajda IJ: Distribution of opiate-like substances in rat tissues. *Neurochem Res*. 1979; 4: 399-410.
4. Quirion R, Finkel MS, Mendelsohn FA, et al.: Localization of opiate binding sites in kidney and adrenal gland of the rat. *Life Sci*. 1983; 33(Suppl 1): 299-302.
5. Stefano GB, Hartman A, Bilfinger TV, et al.: Presence of the mu3 opiate receptor in endothelial cells. Coupling to nitric oxide production and vasodilation. *J Biol Chem*. 1995; 270: 30290-30293.
6. Gupta K, Kshirsagar S, Chang L, et al.: Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. *Cancer Res*. 2002; 62: 4491-4498.
7. Poonawala T, Levay-Young BK, Hebbel RP, et al.: Opioids heal ischemic wounds in the rat. *Wound Repair Regen*. 2005; 13: 165-174.
8. Weber ML HR, Hebbel RP, Gupta K: Opioids promote kidney growth. *J Am Soc Nephrol*. 2005; 16: 387A.
9. Waldhoer M, Bartlett SE, Whistler JL: Opioid receptors. *Annu Rev Biochem*. 2004; 73: 953-990.
10. Wollemann M: Recent developments in the research of opioid receptor subtype molecular characterization. *J Neurochem*. 1990; 54: 1095-1101.
11. Law PY, Loh HH: Regulation of opioid receptor activities. *J Pharmacol Exp Ther*. 1999; 289: 607-624.
12. Gupta K, Stephenson EJ: Existence and modus operandi of opioid receptors in endothelium. In Aird WC (ed.): *The Endothelium: A Comprehensive Reference* [in press]. Cambridge: Cambridge University Press, 2006.
13. Tian W, Zhang Z, Cohen DM: MAPK signaling and the kidney. *Am J Physiol Renal Physiol*. 2000; 279: F593-F604.
14. Gutkind JS: The pathways connecting G protein-coupled receptors to the nucleus through divergent mitogen-activated protein kinase cascades. *J Biol Chem*. 1998; 273: 1839-1842.
15. Fukuda K, Kato S, Morikawa H, et al.: Functional coupling of the opioid receptors to mitogen-activated protein kinase and arachidonate release in Chinese hamster ovary cells. *J Neurochem*. 1996; 67: 1309-1316.
16. Lou L-G, Zhang Z, Ma L, et al.: Nociceptin/orphanin FQ activates mitogen-activated protein kinase in Chinese hamster ovary cells expressing opioid receptor-like receptor. *J Neurochem*. 1998; 70: 1316-1322.
17. Li J-G, Luo L-Y, Krupnick JG, et al.: U50,488H-induced internalization of the human κ opioid receptor involves a β-arrestin- and dynamin-dependent mechanism. κ receptor internalization is not required for mitogen-activated protein kinase activation. *J Biol Chem*. 1999; 274: 12087-12094.
18. Kieffer BL: Recent advances in molecular recognition and signal transduction of active peptides: Receptors for opioid peptides. *Cell Mol Neurobiol*. 1995; 15: 615-635.

19. Welters ID, Fimiani C, Bilfinger TV, et al.: NF-κB, nitric oxide and opiate signaling. *Med Hypotheses.* 2000; 54: 263-268.
20. Li LY, Chang KJ: The stimulatory effect of opioids on mitogen-activated protein kinase in Chinese hamster ovary cells transfected to express mu-opioid receptors. *Mol Pharmacol.* 1996; 50: 599-602.
21. Chuang LF, Killam KF Jr, Chuang RY: Induction and activation of mitogen-activated protein kinases of human lymphocytes as one of the signaling pathways of the immunomodulatory effects of morphine sulfate. *J Biol Chem.* 1997; 272: 26815-26817.
22. Wetzker R, Bohmer FD: Transactivation joins multiple tracks to the ERK/MAPK cascade. *Nat Rev Mol Cell Biol.* 2003; 4: 651-657.
23. Waters C, Pyne S, Pyne NJ: The role of G-protein coupled receptors and associated proteins in receptor tyrosine kinase signal transduction. *Semin Cell Dev Biol.* 2004; 15: 309-323.
24. Belcheva MM, Szucs M, Wang D, et al.: mu-Opioid receptor-mediated ERK activation involves calmodulin-dependent epidermal growth factor receptor transactivation. *J Biol Chem.* 2001; 276: 33847-33853.
25. Chen C, Farooqui M, Gupta K: Morphine stimulates VEGF-like signaling in mouse retinal endothelial cells. Current *Neurovasc Res.* 2006; 3(3), (In press).
26. Kapusta DR, Jones SY, DiBona GF: Renal mu opioid receptor mechanisms in regulation of renal function in rats. *J Pharmacol Exp Ther.* 1991; 258: 111-117.
27. Kapusta DR, Jones SY, DiBona GF: Effects of opioid peptides on neural control of renal function in spontaneously hypertensive rats. *Hypertension.* 1990; 15: 767-773.
28. Kapusta DR, Jones SY, DiBona GF: Opioids in the systemic hemodynamic and renal responses to stress in spontaneously hypertensive rats. *Hypertension.* 1989; 13: 808-816.
29. Mercadante S, Arcuri E: Opioids and renal function. *The Journal of Pain.* 2004; 5: 2-19.
30. Gottlieb HB, Kapusta DR: Endogenous central kappa-opioid systems augment renal sympathetic nerve activity to maximally retain urinary sodium during hypotonic saline volume expansion. *Am J Physiol Regul Integr Comp Physiol.* 2005; 289: R1289-R1296.
31. Shirasaka T, Kunitake T, Kato K, et al.: Nociceptin modulates renal sympathetic nerve activity through a central action in conscious rats. *J Physiol.* 1999; 277: R1025-R1032.
32. Kapusta DR, Dzialowski EM: Central mu opioids mediate differential control of urine flow rate and urinary sodium excretion in conscious rats. *Life Sci.* 1995; 56: PL243-PL248.
33. Kapusta DR, Obih JC: Central kappa opioids blunt the renal excretory responses to volume expansion by a renal nerve-dependent mechanism. *J Pharmacol Exp Ther.* 1995; 273: 199-205.
34. Flores O, Camera LA, Hergueta A, et al.: Role of atrial natriuretic factor, hemodynamic changes and renal nerves in the renal effects of intraperitoneal morphine in conscious rats. *Kidney Blood Press Res.* 1997; 20: 18-24.
35. Kapusta DR, Obih JC: Role of endogenous central opioid mechanisms in maintenance of body sodium balance. *Am J Physiol.* 1995; 268: R723-R730.
36. Wang YX, Clarke GD, Sbacchi M, et al.: Contribution of alpha-2 adrenoceptors to kappa opioid agonist-induced water diuresis in the rat. *J Pharmacol Exp Ther.* 1994; 270: 244-249.
37. Rimoy GH, Bhaskar NK, Wright DM, et al.: Mechanism of diuretic action of spiradoline (U-62066E)—a kappa opioid receptor agonist in the human. *Br J Clin Pharmacol.* 1991; 32: 611-615.
38. Ooi BS, MacCarthy EP, Hsu A: Beta-endorphin amplifies the effect of interleukin-1 on mouse mesangial cell proliferation. *J Lab Clin Med.* 1987; 110: 159-163.
39. Elias AN, Vaziri ND, Maksy M: Plasma beta-endorphin and beta-lipotropin in patients with end-stage renal disease—effects of hemodialysis. *Nephron.* 1986; 43: 173-176.
40. Aronin N, Krieger DT: Plasma immunoreactive beta-endorphin is elevated in uraemia. *Clin Endocrinol (Oxf).* 1983; 18: 459-464.
41. Trelewicz P, Grzeszczak W, Drabczyk R: Serum beta-endorphin in non-dialysed and haemodialysed patients with chronic renal failure. *Int Urol Nephrol.* 1994; 26: 117-123.
42. Flores O, Camera LA, Hergueta A, et al.: Role of atrial natriuretic factor, hemodynamic changes and renal nerves in the renal effects of intraperitoneal morphine in conscious rats. *Kidney Blood Press Res.* 1997; 20: 18-24.
43. Walker LA, Murphy JC: Antinatriuretic effect of acute morphine administration in conscious rats. *J Pharmacol Exp Ther.* 1984; 229: 404-408.
44. Leander JD: A kappa opioid effect: Increased urination in the rat. *J Pharmacol Exp Ther.* 1983; 224: 89-94.
45. Leander JD: Further study of kappa opioids on increased urination. *J Pharmacol Exp Ther.* 1983; 227: 35-41.
46. Sezen SF, Kenigs VA, Kapusta DR: Renal excretory responses produced by the delta opioid agonist, BW373U86, in conscious rats. *J Pharmacol Exp Ther.* 1998; 287: 238-245.
47. Hadrup N, Petersen JS, Praetorius J, et al.: Opioid receptor-like 1 stimulation in the collecting duct induces aquaresis through vasopressin-independent aquaporin-2 downregulation. *Am J Physiol Renal Physiol.* 2004; 287: F160-F168.
48. Johnson JE Jr, White JJ Jr, Walovitch RC, et al.: Effects of morphine on rat kidney glomerular podocytes: A scanning electron microscopic study. *Drug Alcohol Depend.* 1987; 19: 249-257.
49. Patel J, Manjappa N, Bhat R, et al.: Role of oxidative stress and heme oxygenase activity in morphine-induced glomerular epithelial cell growth. *Am J Physiol Renal Physiol.* 2003; 285: F861-F869.
50. Singhal PC, Sharma P, Gibbons N, et al.: Effect of morphine on renomedullary interstitial cell proliferation and matrix accumulation. *Nephron.* 1997; 77: 225-234.
51. Kilcoyne MM, Gocke DJ, Meltzer JI, et al.: Nephrotic syndrome in heroin addicts. *Lancet.* 1972; 1: 17-20.
52. Salomon MI, Poon TP, Goldblatt M, et al.: Renal lesions in heroin addicts. A study based on kidney biopsies. *Nephron.* 1972; 9: 356-363.
53. Atici S, Cinel I, Cinel L, et al.: Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model. *J Biosci.* 2005; 30: 245-252.
54. Michael AF, Keane WF, Raij L, et al.: The glomerular mesangium. *Kidney Int.* 1980; 17: 141-154.
55. Pesce CM, Striker LJ, Peten E, et al.: Glomerulosclerosis at both early and late stages is associated with increased cell turnover in mice transgenic for growth hormone. *Lab Invest.* 1991; 65: 601-605.
56. Wikstrom B, Gellert R, Ladefoged SD, et al.: Kappa-opioid system in uremic pruritus: Multicenter, randomized, double-blind, placebo-controlled clinical studies. *J Am Soc Nephrol.* 2005; 16: 3742-3747.
57. Bosch-Marce M, Poo JL, Jimenez W, et al.: Comparison of two aquaretic drugs (niravoline and OPC-31260) in cirrhotic rats with ascites and water retention. *J Pharmacol Exp Ther.* 1999; 289: 194-201.
58. United States Renal Data System: *USRDS 2000 Annual Data Report.* Bethesda: The National Institutes of Health, National Institute of Diabetes and Digestive Diseases, 2001.