Blood Pressure Regulation by Vasoactive Peptide Genes : Transgenic And Knockout Animal Models

Dowhan Ahn

Department of Physiology, Kosin University College of Medicine, Busan, Korea

Hypertension is a polygenic and multifactorial disease and is intimately related to salt homeostasis. Four important vasoactive peptide systems participate in regulating blood pressure and salt homeostasis. Their interplay is indispensible in many physiologic and pathologic conditions. While the renin-angiotensin and the endothelin systems raise blood pressure by inducing vasoconstriction and sodium retention (or excretion), the kallikrein-kinin and the natriuretic peptide systems reduce blood pressure by eliciting vasodilatation and natriuresis. Gene targeting as well as transgenesis have provided us a lot of information on the biological functions of the genes of these systems. Animal models from these technologies are discussed in relation to blood pressure regulation.

Key Words : Transgenesis, Knockout, Hypertension, Angiotensin, Endothelin, Kinin, Natriretic peptide, Homeostasis

Hypertension is a polygenic and multifactorial disease in which many physiologic blood-pressure regulating systems are involved, unfortunately. In approximately 90% of hypertension the causes are unknown[1]. However, it is well known that long-term regulation of blood pressure is dependent upon renal salt handling, thus blood pressure regulation and salt homeostasis are tightly tuned in some pathologic conditions[2, 3]. Vasoactive peptides are also key regulators of blood pressure, for which they directly modulate vascular tone or indirectly affect renal salt balance[4]. The best-known vasoactive peptides include the renin-angiotensin system, the endothelin system, the kallikrein-kinin system, and the natriuretic peptide system. The former two are related to elevating blood pressure, whereas the latter two are engaged in lowering it.

In vivo functions of these vasoactive peptide systems could be effectively explored in transgenic and knockout animals[5]. Overexpression transgenesis is performed by creating transgenic mice by injecting a gene of interest into pronucleus of one-cell embryo. Knockout study is to use gene targeting technology. Briefly, recombinant ES cells that have targeting vectors consisting of modified gene sequences and a selectable marker are injected into blastocysts, where they can contribute to the development of a chimera. Through back-crossing of chimeric mice and subsequent breeding of the heterozygotes, homozygous null mice are generated. The processes creating trangenic and knockout mice are shown in Fig. 1.

In this communication, I will focus on transgenic and knockout animals created through genetic manipulation of vasoactive peptide genes in relation to hypertension. My intention is to introduce usefulness of such models in hypertension study.

The Renin-Angiotensin System

The renin-angiotensin system (RAS) is one of the best known for the regulation of blood pressure and

Corresponding author : Dowhan Ahn, Department of Physiology, Kosin University College of Medicine, Busan, Korea
Tel : 82-51-990-6415 Fax : 82-51-990-3081
E-mail : dwahn@ns.kosinmed.or.kr

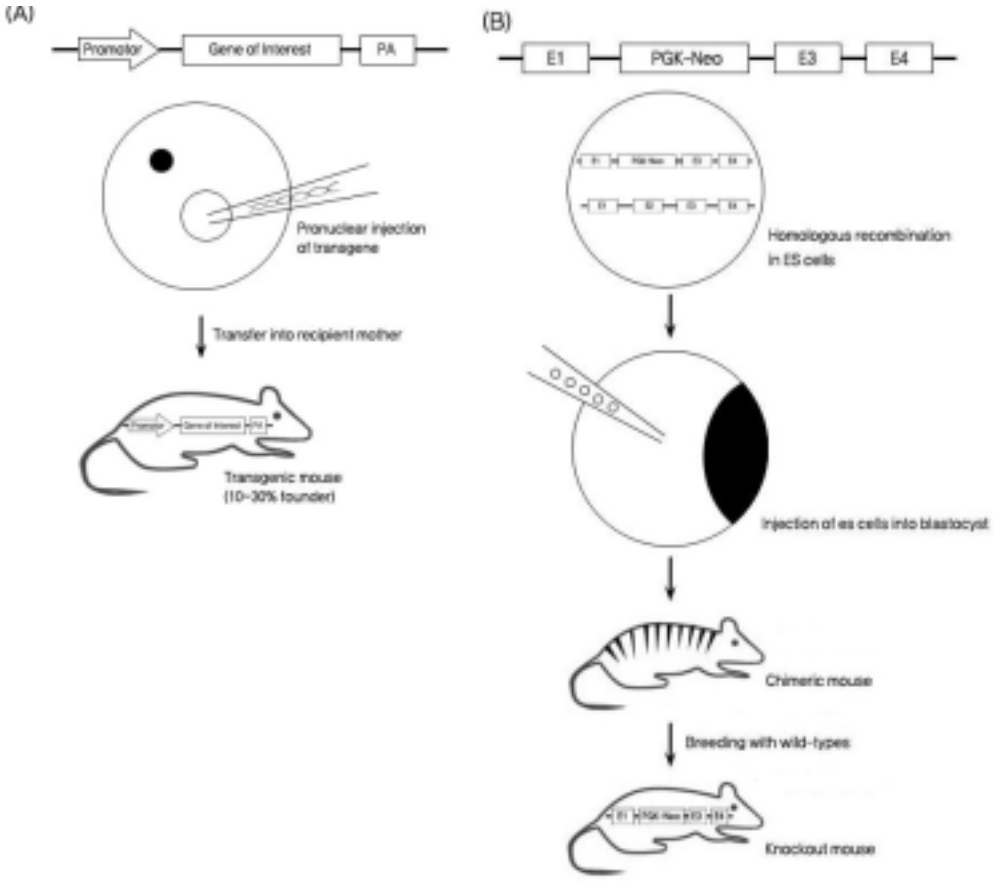

Fig. 1. *Creation of transgenic and knockout mice. (A) The transgene is injected into male pronucleus of one-cell embryo, then transferred into a pseudopregnant mother. (B) The targeting construct containing a selection cassette (PGK-Neo) is electroporated into ES cells. Homologously recombined ES cells are injected into a recipient blastocyst, which is transferred into a pseudopregnant mother. The chimeric mice are back-crossed with wild type mice. PA: polyadenylation site.*

sodium homeostasis. The individual component of this system includes angiotensin (AT), renin, angiotensin-converting enzyme (ACE), and angiotensin receptors. The enzymatic reaction cascade is summarized in Fig. 2. The final product of the RAS system AT II consists of 8 amino acids. In response to AT II, both the renal proximal tubules and intestinal epithelia increase salt and water (re)absorption. Within the adrenal it stimulates aldosterone production, which facilitates sodium reabsorption in the aldosterone sensitive distal nephron (ASDN)[4]. AT II increases the resistance of afferent and efferent arterioles of the kidney as well as peripheral vascular resistance.

Transgenic models

The transgenic mice of both rat angiotensinogen and rat renin under the mouse metallothionein-1 promotor develop hypertension, whereas the blood pressure obtained from the transgenic mice with either rat angiotensinogen or rat renin gene is not different from that of wild-type controls[6]. Similar results were obtained in the hybrid carrying either the human angiotensinogen and human renin genes[7]. These double transgenic mice show an increased plasma AT II and renal lesions exhibited in hypertension. Mullins and colleagues[8] introduced the mouse Ren-2 gene (a C56BL/6 strain has one copy of renin gene (Ren-1c) per haploid genome, and a 129 strain naturally has

two closely linked renin genes (Ren-1d and Ren-2)) into normotensive rats, thus creating a transgenic rat model that expresses high levels of Ren-2 mRNA in many sites. In these rats fulminant hypertension develops between 5 and 10 weeks of age, but kidney and plasma renin levels are suppressed, suggesting low-renin hypertension.

Knockout mice

Homozygous disruption of the angiotensinogen gene not only causes marked hypotension, but also exhibits dilated cardiomyopathy, impaired blood brain barrier function, and severe renal abnormalities including medullary atrophy, arterial wall thickening, and interstitial fibrosis[9-12]. Such abnormalities facilitate an increased mortality in these knockouts. Ishida et al[13] rescued most of the phenotypes of the angiotensinogen null mice by generating a new line of mice, which is produced by crossing the transgenics heterozygous for the mouse angiotensinogen gene under the control of a mouse metallothinein promotor with angiotensinogen null homozygotes. The rescued mice showed comparable values of plasma AT I compared with wild type mice, confirming a definite role of angiotensinogen in blood pressure control. Ren-1c deficient mice show neither detectable levels of plasma renin activity nor plasma AT I, lowered blood pressure 20-30 mmHg less than normal, increased urine and drinking volume, and altered renal morphology as those observed in angiotensinogen-deficient mice[14]. The null mutants, therefore, clearly show an classical role of renin in generating AT I and maintaining blood pressure. On the other hand, Ren-1d knockout mice have decreased blood pressure in females but not in males[15], whereas disruption of the Ren-2 gene does not lead to any changes in blood pressure[16]. These findings suggest that some mouse strains with two renin genes have genetic redundancy. ACE null mice show a marked reduction (-30 mmHg) in blood pressure and maldevelopment of the kidney observed in angiotensinogen knockouts and Ren-1 knockouts[17]. Because testis-specific promotor is located within intron 12 of the ACE gene, these null mutants also show male fertility. Esther et al[18] elegantly created a new kind of ACE null mice (tissue null), which have one-third levels of circulating plasma ACE activity but have no tissue-bound enzyme. The phenotypes of tissue null mice are similar to those of the ACE null mice, i.e. low blood pressure, renal vascular thickening, and a urine concentrating defect. But they show normal development of the kidney, hence retain normal renal function. AT-1a receptor knockout is moderately hypotensive and has a milder degree of renal abnormalities than angiotensinogen and ACE- deficient mice[19]. Normal blood pressure and renal function are evident in mice lacking the AT-1b receptor[20]. Therefore, it is likely that AT-1a is a primary receptor for AT II signaling. AT-1a/AT-1b double knockouts show a similar phenotype of angiotensinogen and ACE-deficient mice[21]. In contrast to the lowering of blood pressure in AT-1a knockout mice, a modest elevation in blood pressure was reported in mice lacking the AT-2 receptor, suggesting a counteractive role of AT-2 receptor in AT-1 receptor-mediated signaling of AT II[22]. Collectively, the RAS is proven to be indispensible for physiologic regulation of blood pressure and for proper development of the kidney.

The Kallikrein-Kinin System

The main components of the kallikrein-kinin system (KKS) are the substrate kininogen, the enzyme kallikrein, and the effector kinin (lys-bradykinin or bradykinin). The rat and mouse produce bradykinin and all other species generate lys-badykinin (or kallidin). Bradykinin is consists of 9 amino acids and kallidin has 10 amino acids with lysine added to bradykinin. The reaction cascade is summarized in Fig. 2. The kinin binds to two types of receptors, B1 and B2. The B2 receptor is widely distributed in tissues and is stimulated by both bradykinin and

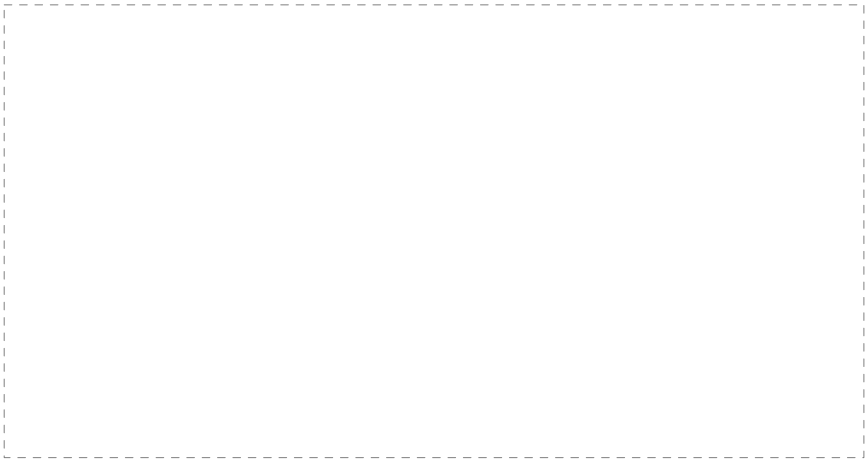

Fig. 2. *The simplified enzymatic cascades of the vasoactive peptides : the renin-angiotensin system, the kallikrein-kinin system, the natriuretic peptide system, and the endothelin system. ACE : angiotensin-converting enzyme. ATR : angiotensin receptor. B : bradykinin receptor. NEP : neutral endopeptidase. ANP : atrial natriuretic peptide. NPR : natriuretic peptide receptor. ET: endothelin. ECE : endothelin-converting enzyme. ETR : endothelin receptor.*

lys-bradykinin, while the B1 receptor is highly inducible by inflammatory mediators and binds to the fragments cleaved by kininase I[4]. Intravascular injection of bradykinin causes a decrease in total peripheral vascular resistance and systemic blood pressure, indicating a potent vasodilator[23]. The local infusion of bradykinin into kidney increases sodium and water excretion[24]. However, the pharmacological blokcade of the B2 receptor induces arterial hypertension with sodium and water retention[25]. These results clearly indicate that the KKS participates in the regulation of blood pressure and fluid homeostasis.

Transgenic models

Transgenic mice overexpressing human tissue kallikrein under the promotor of the mouse metallothionein and albumin genes show a sustained reduction in blood pressure throughout their life sapn, indicating that they lack sufficient compensatory mechanisms to reverse the the hypotensive effect of kallikrein[26]. Wang et al[27] created transgenic mice that harbor the human B2 receptor transgene. The mice also show chronic hypotension and enhanced renal functions such as renal plasma flow and glomerular filtration rate[28]. Overexpression of rat B1 receptor shows baseline normotension but induces hypertension and inflammatory responses to a B1 agonist[29].

Knockout models

Kininogen-deficient mice have not been developed (probably because of the unexpected existance of two genes encoding kallikrein sensitive kininogen). Instead, Brown Norway-Katholiek rats, which have very low levels of low molecular weight kininogen in plasma and do not export synthesized kininogen, are used as a kininogen-deficient model. The rats are normotensive on a normal diet but show hypertensive responses when challenged with salt loading[30]. Tissue kallikrein-deficient mice present a reduced cardiac function, with no changes in blood pressure despite a decrease in renal and urinary kallikrein activity, suggesting that a low renal kallikrein synthesis is not a primary cause of high blood pressure but rather a consequence of hypertension[31]. The B2 receptor knockout is reported to show an elevated blood pressure under basal condition, an exaggerated pres-

sure response to dietary salt loading and an dilated cardiomyopathy[32, 33]. However, Milia et al[34] have found that the basal blood pressure is similar between the wild-type and B2 receptor knockouts and that high salt intake does not affect the blood pressure in B2 receptor knockouts, which suggests that an adaptation to the loss of B2 receptor function may occur. Therefore, the question as to whether or not B2 receptor plays a significant role in hypertension under basal condition and in salt-sensitive hypertension remains to be answered. B1 receptor gene knockout mice are normotensive and show altered inflammatory responses and hypoalgesia[35].

The Natriuretic Peptide System

The natriuretic peptide system is composed of atrial (ANP), brain (BNP), and C-type (CNP) natriuretic peptides. The reaction cascade is summarized in Fig. 2. ANP contains 28 amino acids and is the predominant member of the three. The physiologic action of ANP includes the reduction of intravascular volume and inhibition of sodium reabsorption in the renal medullary collecting duct[4]. The acute hypotensive effect of ANP is mediated primarily by a decrease in cardiac output, which is due to a reduction in total blood volume[36]. The chronic effects of ANP on blood pressure and salt balance are hampered by lack of suitable natural models of ANP-induced diseases and the unavailability of selective pharmacological receptor antagonists[37]. The actions of BNP are quantitatively similar to those of ANP. In the kidney alternate processing of pro-ANP generates a 32-amino acid peptide urodilatin, which primarily regulates sodium transport in the distal nephron[38]. The biological function of the natriuretic peptides is mediated by natriuretic peptide receptor-A (NPR-A) and NPR-B. NPR-C is generally thought to be a clearance receptor.

Transgenic models

Steinhelper et al[39] generated ANP-overexpressing trangenic mice, in which a fusion gene comprising the mouse transthyretin promoter and mouse atrial natriuretic factor structural sequences was designed so as to target hormone expression to the liver. The transgenic animals manifest 5-10 fold elevation of plasma ANP and life-long hypotension relative to nontransgenic controls. This difference in arterial pressure is not accompanied by significant changes in several other physiological parameters such as glomerular filtration rate. Therefore, it is likely that the hypotensive effect of ANP is mediated by direct cardiovascular action of the hormone.

Knockout models

The ANP gene knockout mice are hypertensive even on diets containing very little salt as well as normal salt diets[40]. This is due to elevated total peripheral resistance because the null mutants have comparable levels of heart rate, cardiac output, and stroke volume compared with the wild-type[41]. They also show salt-sensitive hypertension[42]. It seems that ANP acts to lower blood pressure and that under conditions of normal salt intake it is not essential for normal salt balance, but is one of several natriuretic mechanisms. NPR-A knockout models have been developed by two independent groups. The two models present a significant and quantitatively similar increase in basal arterial pressure compared with their wild-type controls, thereby indicating a fundamental role of the NPR-A receptor in mediating the chronic hypotensive effect of ANP. However, the null mice from Oliver et al[43] develop high mortality rates at 6 months of age in association with severe cardiac hypertrophy and sudden death, whereas the knockout mice from Lopez et al[44] do not show such changes in cardiac morphology and mortality. The underlying

cause for the differences between the two mutant types is not known. Interestingly, the knockout mice from the Lopez group show the phenotype of salt-resistant hypertension, i.e. the blood pressure remains elevated and unchanged in response to either minimal or high salt diets. Aldosterone and ANP concentrations are not affected in these null mutants. NPR-C knockouts show a slight increase in blood pressure, but unexpectedly have skeletal deformities[45].

The Endothelin System

The endothelins (ET) have three isopeptides, ET-1, ET-2, and ET-3, all consisting of 21-amino acids. Among these the actions of ET-1 are best known. ET-1 is the most potent vasoconsrictor studied in vitro, 30-50 times more potent than norepinephrine or angiotensin II. The important sites of ET-1 generation are endothelial cells and the kidney medulla, where ET-1 is predominently secreted into the basolateral side. Fig 2 shows the processes of ET production. ET-1 exerts its major effects through activation of A type (ETRA) or B type (ETRB) receptor. The ETRA is preferentially expressed in vascular smooth muscle-cells and induces vasoconstriction and mitogenesis, whereas ETRB is widely expressed in endothelial cells and renal epithelium and mediates vasodilation and the effects of natriuresis and diuresis [46].

Transgenic models

Overexpression of the human ET-1 gene under the control of its natural promoter is associated with an age-dependent development of renal interstitial fibrosis and glomerulosclerosis, leading to a progressive decrease in glomerular filtration rate without alterations of blood pressure[47]. This blood pressure-independent fibrotic remodeling of the kidney occurs despite a rather low overexpresion rate of ET-1. A similar observation has also been made in transgenic rats overexpressing the human ET-2 gene under control of its own promoter[48]. However, the extent of kidney fibrosis was less pronounced and mainly restricted to the glomeruli. This is most probably due to the preferential expression of the transgene within the glomeruli in ET-2 transgenic rats, whereas the transgene is ubiquitously expressed within the entire kidney of ET-1 transgenic mice. The absence of hypertension in these transgenic animal models of the ET system was unexpected, since an i.v. injection of ET-1 or ET-2 caused a very sustained vasoconstriction. The lack of hypertension is probably due to the compensatory activation of strong vasodilator systems such as the NO system. Amiri et al[49] targeted expression of the human ET-1 gene to enodothelium by using Tie2 promotor in order to directly test the role of endothelium-derived ET-1. The resulting transgenic mice exhibited 8-fold increase in ET-1 plasma levels than wild type mice but no significant elevation in blood pressure.

Conventional Knockout models

Homozygous ET-1 knockout mice die immediately after birth due to craniopharyngeal malformations. Unexpectedly, heterozygous ET-1 knockout mice show an elevated blood pressure with higher Pco_2 and lower Po_2 compared to wild-types, suggesting that ET-1 is involved in cardiovascular and respiratory homeostasis[50]. An elevated blood pressure is also detected in ETRB knockout mice, but the major phenotype in these mice is the absence of enteric ganglionic cells, leading to a congenital megacolon with early postnatal death. To resolve this perinatal problem, Gariepy et al[51] rescued ETRB-deficient rats by selective expression of ETRB receptors in intestinal ganglion cells. This can be achieved by cross-breeding the natural ETRB-deficient rats with the transgenic rats overexpressing the ETRB gene under the control of the dopamine β-hydroxylase gene promoter, which directs ETRB

expression to enteric nerve cells. The rescued ETRB rats exhibit increased circulating ET-1 and are hypertensive. The hypertension is salt-sensitive, indicating that the regulation of salt excretion in the kidneys is partially ETRB-dependent. ETRA knockout mice are characterized by severe craniofacial deformities and defects in the cardiovascular outflow tract, so they die shortly after birth[52]. ECE-1 null mice exhibit craniofacial and cardiac abnormalities virtually identical to the defects seen in ET-1 and ETRA-deficient mice[53]. Therefore, null micefrom all components of the ET system show lethal phenotypes, hence it is actually impossible to examine the effects of ET on hypertension and salt homeostasis.

Conditional knockout models

In order to circumvent these embryonic lethalities, a conditional knockout strategy has been devised, in which gene targeting can be spatially and temporally regulated[54, 55]. The approach involves Cre/loxP recombination. Cre recombinase is an enzyme that is produced by bacteriophage P1 and is not normally present in mammalian cells. Cre mediates recombination at 34-base pair sequences, called loxP. If two loxP sites are inserted in the same orientation into the DNA flanking a sequence of interest, then Cre will mediate recombination between the loxP sites. The DNA segment between two loxP sites will be excised, leaving behind a single loxP site in the original DNA. Cre/loxP recombination, therefore, can be used to create deletions at any desired location in the genome. To produce tissue-specific gene knockouts, two strains of mice are required. One strain expresses Cre recombinase under the control of the promoter of a tissue-specific gene. Typically, this strain is produced by conventional transgenic methods described previously. The second mouse strain contains two loxP sites flanking the DNA segment to be excised. The loxP sites are inserted into introns flanking an essential exon(s) of the gene of interest by using a conventional gene targeting technique. Since the loxP sites usually do not affect gene expression, the resulting “floxed” mice have a wild-type phenotype. Next, mating of the two mouse lines should result in Cre-mediated gene deletion only in those cells in which the promotor is active. Fig. 3 briefly depicts the processes of conditional gene targeting based on Cre/loxP technology.

Ahn et al[56] has recently developed renal collecting duct-specific knockouts of ET-1 by using a Cre/loxP technique. The transgenic mice with aquaporin-2

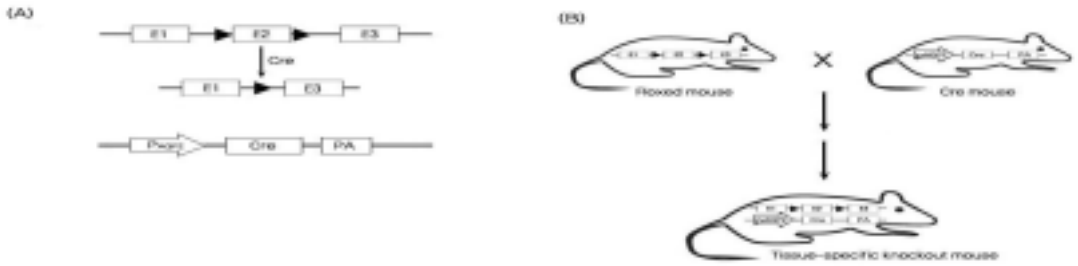

Fig. 3. *Conditional gene targeting by Cre/loxP recombination system. (A). Cre recombinase excises the loxP-flanked E2 (exon 2). Excised DNA is degraded by cellular nucleases. The promotor of AQP2 will drive Cre recombinase to the collecting duct. (B). A floxed mouse and a Cre mouse are respectively generated by the technique described in Fig. 1. Mating of the two mice results in deletion of E2 in the collecting duct only.*

promotor-driven Cre recombinase, in which Cre is uniquiely expressed in the collecting duct, were mated with floxed mice which have loxP-flanked exon 2 of ET-1 gene. The resulting double transgenic mice are devoid of ET-1 in the collecting duct and grow normally. Kidney structure and function of these mice are not different from those of wild type mice. Plasma ET-1, renin, and urinary aldosterone excretion are not changed, but urinary ET-1 excretion is moderately decreased in the knockouts. They are hypertensive and the hypertension is more aggravated by a high salt diet, suggesting salt-sensitive hypertension. Thus, it is demonstrated that renal ET-1 is an important physiologic regulator of systemic blood pressure and sodium handling, possibly through ETRB. Kidney-specific or endothelium-specific knockouts of ETRA or ETRB gene have not been reported yet.

Conclusion

In vivo physiologic function of a system can be clearly understood in naturally-occurring model animals or those created through genetic manipulation of the genes of the system. Transgenic or knockout mice models of the vasoactive peptide system (VPS) have been widely used to study whether the VPS is actively involved in blood pressure regulation and salt homeostasis. From these models it became clear that the VPS plays an important role in blood pressure regulation and salt homeostasis. But limitations of such models also arise because of undesirable and uncontrollable effects on other systems. Tissue-specific gene targeting can be an alternative, although laborious and time-consuming. It should also be born in mind that many other physiologic systems such as NO and prostanoids also interact with them for the regulation of blood pressure and/or salt homeostasis.

References

1) Ganong WF : Cardiovascular disorders : Vascular disease. In: *Pathophysiology of Disease*, 4th ed., edited by McPhee SJ, Lingappa VR, Ganong WF, Seoul, McGraw-Hill, 2003, p318-323
2) Guyton AC : Blood pressure control : special role of the kidneys and body fluids. *Science* **252:**1813-1816, 1991
3) Kevin M, O'shaughnessy, Karet FE : Salt handling and hypertension. *J Clin Invest* **113:**1075-1081, 2004
4) Candido R, Burrell LM, Jandeleit-Dahm KA, Cooper ME : Vasoactive peptides and the kidney. In : *The Kidney*, 7th ed., edited by Brenner BM, Phildelphia, WB Saunders, 2004, p663-726
5) Bockamp E, Maringer M, Spangenberg C, Fees S, Fraser S, Eshkind L, Oesch F, Zabel B : Of mice and models: improved animal models for biomedical research. *Physiol Genomics* **11:**115-132, 2002
6) Ohkubo H, Kawakami H, Kakehi Y, Takumi T, Arai H, Yokota Y, Iwai M, Tanabe Y, Masu M, HataJ, Iwao H, Okamoto H, Yokoyama M, Nomura T, Katsuki M, Nakanishi S : Generation of transgenic mice with elevated blood pressure by introduction of the rat renin and angiotensinogen genes. *Proc Natl Acad Sci U S A* **87:**5153-5157, 1990
7) Merrill DC, Thompson MW, Carney CL, Granwehr BP, Schlager G, Robillard JE, Sigmund CD : Chronic hypertension and altered baroreflex responses in transgenic mice containing the human renin and human angiotensinogen genes. *J Clin Invest* **97:** 1047-55, 1996
8) Mullins JJ, Peters J, Ganten D. Fulminant hypertension in transgenic rats harboring the mouse Ren-2 gene. *Nature* **344:**541-544, 1990
9) Tanimoto K, Sugiyama F, Goto Y, Ishida J, Takimoto E, Yagami K, Fukamizu A, Murakami K: Angiotensinogen-deficient mice with hypotension. *J Biol Chem* **269:**31334-31337, 1994
10) Kim HS, Krege JH, Kluckman KD, Hagaman JR, Hodgin JB, Best CF, Jennette JC, Coffman TM, Maeda N, Smithies O : Genetic control of blood pressure and the angiotensinogen locus. *Proc Natl Acad Sci USA* **92:**2735-2739, 1995
11) Kakinuma Y, Hama H, Sugiyama F, Yagami K, Goto K, Murakami K, Fukamizu A : Impaired blood-brain barrier function in angiotensinogen-deficient mice. *Nat Med* **4:**1078-1080, 1998
12) Walther T, Steendijk P, Westermann D, Hohmann C, Schulze K, Heringer-Walther S, Schultheiss HP, Tschope C : Angiotensin deficiency in mice leads to dilated cardiomyopathy. *Eur J Pharmacol* **493:**161-165, 2004

13) Ishida J, Sugiyama F, Tanimoto K, Taniguchi K, Syouji M, Takimoto E, Horiguchi H, Murakami K, Yagami K, Fukamizu A : Rescue of angiotensinogen-knockout mice. Biochem Biophys Res Comm 252:610-616, 1998
14) Yanai K, Saito T, Kakinuma Y, Kon Y, Hirota K, Taniguchi-Yanai K, Nishijo N, Shigematsu Y, Horihuchi H, Kasuya Y, Sugiyama F, Yagami K, Murakami K, Fukamizu A : Renin-dependent cardiovascular functions and renin-independent blood-brain barrier functions revealed by renin-deficient mice. *J Biol Chem* **275:**5-8, 2000
15) Clark AF, Sharp MG, Morley SD, Fleming S, Peters J, Mullins JJ. Renin-1 is essential for normal renal juxtaglomerular cell granulation and macula densa morphology. *J Biol Chem* **272:**18185-90, 1997
16) Sharp MG, Fettes D, Brooker G, Clark AF, Peters J, Fleming S, Mullins JJ : Targeted inactivation of the Ren-2 gene in mice. *Hypertension* **28:**1126-1131, 1996
17) Cole JM, Xiao H, Adams JW, Disher KM, Zhao H, Bernstein KE : New approaches to genetic manipulation of mice: Tissue-specific expression of ACE. *Am J Physiol* **284:**F599-607, 2003
18) Esther CR, Marino EM, Howard TE, Machaud A, Corvol P, Capecchi MR, Bernstein KE : The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J Clin Invest* **99:**2375-2385, 1997
19) Sugaya T, Nishimatsu S, Tanimoto K, Takimoto E, Yamagishi T, Imamura K, Goto S, Imaizumi K, Hisada Y, Otsuka A, Uchida H, Sugiura M, Fukuta K, Fukamizu A, Murakami K : Angiotensin II type 1a receptor-deficient mice with hypotension and hyperreninemia. *J Biol Chem* **270:**18719-18722, 1995
20) Chen XM, Li WG, Yoshida H, Tsuchida S, Nishimura H, Takemoto F, Okubo S, Fogo A, Matsusaka T, Ichikawa I : Targeting deletion of angiotensin type 1B receptor gene in the mouse. *Am J Physiol* **41:**F299-304, 1997
21) Tsuchida S, Matsusaka T, Chen X, Okubo S, Miimura F, Nishimura H, Fogo A, Utsunomiya H, Inagami T, Ichikawa I : Murine double nullizygotes of the angiotensin type 1A and 1B receptor genes duplicate severe abnormal phenotypes of angiotensinogen nullizygotes. *J Clin Invest* **101:**755-760, 1998
22) Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Miimura F, Ichikawa I, Hogan BL, Inagami T : Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. *Nature* **377:**745-750, 1995
23) Regoli D, Barabe J : Pharmacology of bradykinin and related kinins. *Pharmacol Rev* **32:**1-46, 1980
24) Mattson DL, Cowley AW Jr. Kinin actions on renal papillary blood flow and sodium excretion. *Hypertension* **21:**961-965, 1993
25) Tornel J, Madrid MI, Garcia-Salom M, Wirth KJ, Fenoy FJ : Role of kinins in the control of renal papillary blood flow, pressure natriuresis, and arterial pressure. Circ Res 86:589-95, 2000
26) Chao J, Chao L : Functional analysis of human tissue kallikrein in transgenic mouse models. *Hypertension* **27:**491-494, 1996
27) Wang DZ, Chao L, Chao J : Hypotension in transgenic mice overexpressing human bradykinin B2 receptor. Hypertension 29:488-493, 1997
28) Wang D, Yoshida H, Song Q, Chao L, Chao J : Enhanced renal function in bradykinin B(2) receptor transgenic mice. *Am J Physiol* **278:**F484-491, 2000
29) Ni A, Yin H, Agata J, Yang Z, Chao L, Chao J : Overexpression of kinin B1 receptors induces hypertensive response to des-arg9-bradykinin and susceptibility to inflammation. *J Biol Chem* **278:**219-225, 2003
30) Majima M, Yoshida O, Mihara H, Muto T, Mizogami S, Kuribayashi Y, Katori M, Ohishi S : High sensitivity to salt in kininogen-deficient brown Norway-Katholiek rats. Hypertension **22:**705-714, 1993
31) Meneton P, Bloch-Faure M, Hagege AA, Ruetten H, Huang W, Bergaya S, Ceiler D, Gehring D, Martins I, Salmon G, Boulanger CM, Nussberger J, Crozatier B, Gasc JM, Heudes D, Bruneval P, Doetschman T, Menard J, Alhenc-Gelas F : Cardiovascular abnormalities with normal blood pressure in tissue kallikrein-deficient mice. *Proc Natl Acad Sci USA* **98:**2634-2639, 2001
32) Alfie ME, Sigmon DH, Pomposiello SI, Carretero OA : Effect of high salt intake in mutant mice lacking bradykinin-B2 receptors. *Hypertension* **29:**483-487, 1997
33) Cervenda L, Herrison-Bernard LM, Dipp S, Primrose G, Imig JD, El-Dahr SS : Early onset salt-sensitive hypertension in bradykinin B2 receptor null mice. *Hypertension* **34:**176-180, 1999
34) Milia AF, Gross V, Plehm R, Silva JAD, Bader M, Luft FC : Normal blood pressure and renal function in mice lacking the bradykinin B2 receptor. *Hypertension* **37:**1473-1497, 2001
35) Pesquero JB, Araujo RC, Heppenstall PA, Stucky CL, Silva JA, Walther T, Oliveira SM, Pesquero JL, Paiva ACM, Calixto JB, Lewin GR, Bader M : Hypoalgesia and altered inflammatory responses in mice lacking kinin B1 receptors. *Proc Natl Acad Sci USA* **97:**8140-8045, 2000
36) Lee RW, Goldman S : Mechanism for decrease in cardiac output with atrial natriuretic peptide in dogs. *Am J Physiol* **256:**H760-765, 1989
37) Melo LG, Steinhelper ME, Pang SC, Tse Y,

Ackermann U : ANP in regulation of arterial pressure and fluid-electrolyte balance: lessons from genetic mouse models. *Physiol Genomics* **3:**45-58, 2000

38) Goetz KL: Renal natriuretic peptide (urodilatin) and atriopeptin: evolving concepts. *Am J Physiol* **261:** F921-932, 1991

39) Steinhelper ME, Cochrane KL, Field LJ : Hypotension in transgenic mice expressing atrial natriureic factor fusion genes. *Hypertension* **16:**301-307, 1990

40) John SWM, Veress AT, Honrath U, Chong CK, Peng L, Smithies O, Sonnenberg H : Blood pressure and fluid-electrolyte balance in mice with reduced or absent ANP. *Am J Physiol* **271:**109-114, 1996

41) Melo LG, Veress AT, Ackermann U, Pang SC, Flynn TG, Sonnenberg H : Chronic hypertension in ANP knockout mice: contribution of peripheral resistance. *Regul Pept* **79:**109-115, 1999

42) Melo LG, Veress AT, Chong CK, Pang SC, Flynn TG, Sonnenberg H : Salt-sensitive hypertension in ANP knockout mice: potential role of abnormal plasma renin activity. *Am J Physiol* **274:**R255-261, 1998

43) Oliver PM, Fox JE, KimR, Rockman HA, Kim HS, Reddick RL, Pandey KN, Milgram SL, Smithies O, Maeda N : Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriureic peptide receptor A. *Proc Natl Acad Sci USA* **94:**14730-14735, 1997

44) Lopez MJ, Wong SK, Kishimoto I, Dubois S, Mach V, Friesen J, Garders DL, Beuve A : Salt-resistant hypertension in mice lacking the guanylyl cyclase-A receptor for atrial natriuretic peptide. *Nature* **378:**65-68, 1995

45) Matsukawa N, Grzesik WJ, Takahashi N, Pandey KN, Pang S, Yamauchi M, Smithies O : The natriuretic peptide clearance receptor locally modulates the physilogical effects of the natriuretic peptide system. *Proc Natl Acad Sci USA* **96:**7403-7408, 1999

46) Simonson MS : Endothelins: Multifunctional renal peptides. *Physiol Rev* **73:**375-411, 1993

47) Hocher B, Thone-Reineke C, Rohmeiss P, Schmager F, Slowinski T, Burst V, Siegmund F, Quertermous T, Bauer c, Neumayer HH, Schleuning WD, Therting F : Endothelin-1 transgenic mice develop glomerulosclerosis, interstitial fibrosis, and renal cysts but not hypertension. *J Clin Invest* **99:** 1380-1389, 1997

48) Hocher B, Liefeldt L, Thone-Reineke C, Orzechowski HD, Distler A, Bauer C, Paul M : Characterization of the renal phenotype of transgenicrats expressing the human endothelin-2 gene. *Hypertension* **28:**196-201, 1996

49) Amiri F, Virdis A,Neves MF, Iglarz M, Seidah NG, Touyz RM, Reudelhuber TL, Schiffrin EL : Endothelium-restricted overexpression of human endothelin-1 cause vascular remodeling and endothelial dysfunction. *Circulation* **110:**2233-2240, 2004

50) Kuwaki T, Ling GY, Onodera M, Ishii T, Nakamura A, Ju KH, Cao WH, Kumada M, Kurihara H, Kuridara Y, Yazaki Y, Ohuchi T, Yanagisawa M, Fukuda Y : Endothelin in the central control of cardiovascular and respiratory functions. *Clin Exp Pharmacol Physiol* **26:**989-994, 1999

51) Gariepy CE, Williams SC, Richardson JA, Hammer RE, Yanagisawa M : Transgenic expression of the endothelin-B receptor prevents congenital intestinal aganglionosis in a rat model of hirschsprung disease. *J Clin Invest* **102:**1092-1101, 1998

52) Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, Kumada M, Hammer RE, Yanagisawa M : Cranial and cardiac neural crest defeats in endothelin-A receptor-deficient mice. *Development* **125:**813-824, 1998

53) Yanagisawa H, Yanagisawa M, Kapur RP, Richardson JA, Williams SC, Clouthier DE, Wit D, Emoto N, Hammer RE : Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. *Development* **125:**825-836, 1998

54) Stricklett PK, Nelson RD, Kohan DE : The Cre/loxP system and gene targeting in the kidney. Am J Physiol 276:F651-657, 1999

55) Kwan KM : Conditional alleles in mice: Practical considerations for tissue-specific knockouts. *Genesis* **32:**49-62, 2002

56) Ahn D, Ge Y, Stricklett PK, Gilll P, Taylor D, Hughes AK, Yanagisawa M, Miller L, Nelson RD, Kohan DE : Collecting duct-specific knockout of endothelin-1causes hypertension and sodium retention. *J Clin Invest* **114:**504-511, 2004