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TRANSGENIC RAT MODELS OF VASOPRESSIN OVEREXPRESSION

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ABSTRACT

Vasopressin has an important role in water metabolism and its impairment induces some clinical disorders such as diabetes insipidus or syndrome of inappropriate antidiuresis (SIAD). SIAD is caused by the overproduction of vasopressin which induces diluting hyponatremia. The accurate diagnosis and appropriate therapy have not settled up to date because its pathophysiology is very complicated. It is meaningful to develop a rat model of SIAD in which human vasopressin gene is overexpressed in order to analyze pathophysiological changes. Several models transgenic for vasopressin including us had been generated. The transgenic rats provide a useful model to investigate various pathophysiological changes resulting from the oversecretion of vasopressin. Some interesting results based on these animal models are reviewed.

Key Words: vasopressin, V2 receptor, transgenic rat, downregulation

Biosynthesis, transport and release of vasopressin:

Arginine vasopressin, a nine-amino acid neuropeptide, acts as the antidiuretic hormone and indicates various functions such as maintenance of blood pressure, response to stress, regulating circadian rhythm[1], and behavior[2]. Vasopressin is synthesized in hypothalamus nuclei including the supraoptic nucleus (SON), paraventricular nucleus (PVN), and suprachiasmatic nucleus (SCN). Vasopressin matures from preprovasopressin which consists of the signal peptide, vasopressin, neurophysin (NP), and glycoprotein domain. Preprovasopressin is encoded by the vasopressin gene on chromosome 20 which has three exons[3]. The first exon encodes the signal peptide, vasopressin, and the N-terminal region of NP. The second exon encodes the central region of NP, and the third exon encodes the C-terminal region of NP and the glycoprotein domain[4]. Preprovasopressin is processed in secretory vesicles during axonal transport from the hypothalamus to the posterior pituitary. NP is the carrier protein of vasopressin and protects vasopressin from proteolytic degeneration during axonal transport[5].

Magnocellular neurons in SON and PVN project to the posterior pituitary, where vasopressin is released into the blood in response to hyperosmolar and/or hypovolemic stimuli[6]. It acts on the adenylate cyclase-coupled vasopressin V2 receptor (V2R) in the renal collecting ducts[7] and increases water reabsorption from the urine through a water channel, aquaporin 2 (AQP2)[8], by stimulating its shuttling from intracellular vesicle into the apical plasma membrane and its synthesis.

Parvocellular neurons in PVN project to the median eminence, where vasopressin is released into pituitary portal vessels in response to stress simultaneously with corticotropin-releasing

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hormone(CRH)[9]. Endogenous vasopressin potentiates CRH-stimulated ACTH secretion and plays a physiologically significant role in regulating CRH-stimulated ACTH and cortisol secretion in man.

Clinical disturbances of vasopressin secretion:

Vasopressin deficiency results in central diabetes insipidus with polyuria and polydipsia. There is a genetic animal model, the Brattleboro rat, which fails to produce sufficient vasopressin[10]. In the Brattleboro rats, the gene for the vasopressin precursor lacks a single G residue in the coding region. The mutation gives rise to an open reading frame predicting a hormone precursor having a different C-terminus. The Brattleboro rats have applied to be very useful for studying the pathophysiology of vasopressin deficiency[11].

In humans, familial central diabetes insipidus (FDI) is caused by a deficiency of vasopressin and transmitted as an autosomal dominant trait. The possibility that a mutation in the vasopressin gene may be the basis of FDI had been suggested by indirect observations. We reported a single base substitution in exon 2 of the vasopressin gene in a Japanese FDI pedigree, which results in an amino acid substitution in the NP of the vasopressin precursor[12]. Subsequently, we identified a mutation in exon 1 that predicts the change at the C-terminal of the signal peptide[13]. Then, we have analyzed the vasopressin gene in four Japanese FDI pedigrees and identified four novel mutations in exon 2. One of the mutations predicts a premature termination, and others result in a single amino acid substitution or deletion[14,15]. More than 30 FDI pedigrees including our cases have been reported to date[16]. Interestingly, almost all mutations except the signal peptide mutation are located in exon 2 or 3, suggesting that the important role of the NP molecules. Moreover, all patients are heterozygous for the mutations, indicating that some mechanisms may be involved in the abolishment of vasopressin production by the normal allele. The precise mechanisms of how these abnormalities of vasopressin precursor cause impairment synthesis of vasopressin have not yet been revealed. To investigate the fundamental aspect of the disorder, AtT20 cells were transfected with each mutant vasopressin cDNA. Transport of vasopressin precursor in these transfected cells was significantly impaired compared to control, suggesting the following possibilities: 1. The intracellular transport of an abnormal vasopressin precursor might be impaired by its conformational changes. Accumulated abnormal precursor may result in the cell degeneration of magnocellular neurons. 2. The abnormal NP generated through posttranslational processing of the abnormal precursor might lack the physiologic functions.

On the other hand, in some clinical circumstances, inappropriately high plasma vasopressin results in free-water retention and diluted hyponatremia. Chronic oversecretion of vasopressin causes the syndrome of inappropriate antidiuresis (SIAD) manifesting hypervolemic hyponatremia in humans, however, the pathophysiology has not been well elucidated[17,18]. An animal model of SIAD would be a useful tool to investigate the pathophysiologic changes resulting from chronic hyponatremia. In recent years, the technique of pronuclear injection can be used to incorporate a foreign gene into the genome of a host animal[19]. This technique provides the opportunity to study problems of gene regulation and gene expression in a mammalian by application of recombinant DNA technology.

Vasopressin overexpressing transgenic rat:

The transgene technology has been utilized to produce animals that overexpress vasopressin to explore whether chronic oversecretion of vasopressin would cause SIAD together with alterations in the neuroendocrine systems involving stress response, memory, and circadian rhythm. The first model was reported by Murphy *et al.*[20] They used transgenic mice to study the activity of a hybrid oncogene made up of 1.25 kb of 5' upstream sequences, derived from the bovine vasopressin gene, promoting the expression of the large T-antigen coding sequences of the early region of simian virus 40. Their observations suggest that the specificity of vasopressin gene expression normally results from an interaction between several regulatory elements.

After their first report, several genetic models of vasopressin oversecretion using transgenic technique were developed. Waller *et al.* reported that rat transgenic for 5-VCAT-3 (rat vasopressin-CAT fusion gene flanked by 5 kb of upstream and 3kb of downstream VP gene sequence) expressed tagged rat vasopressin polypeptide in hypothalamic neurons[21]. They reported that a transgenic system facilitates monitoring of a central peptidergic system from transcription to the storage and release of the mature secretory product. Another model was a mice transgenic for the rat vasopressin gene[22]. Expression of the rat vasopressin gene was demonstrated by Southern blotting and resulted in increased amounts of vasopressin in hypothalamus and frontotemporal brain cortex. Secretion of vasopressin from the neurohypophysial system results in an increased concentration of the plasma hormone. Although chronic hypersecretion of vasopressin was seen, 24-hour urine volume and osmolality did not show any evidence of increased antidiuretic hormone action on the kidney, so that, under basal conditions, the water balance in the animals is unaffected. Interestingly, these mice showed enhanced attention and alertness, supporting earlier observations on the effects of vasopressin on behavior and cognitive function.

To be a useful model of gene expression, the transgenes should include important cis-acting promoter and enhancer elements in addition to the structural gene[23]. An 8.2 kb of rat vasopressin gene fragment that has 3 kb each of 5' and 3' ends of untranslated region (UTR), has been used to develop a line of transgenic mice[24]. Using a PCR technique, the rat vasopressin transgene was shown to have tissue-specific mRNA expression in the hypothalamus, temporal lobe, parietal cerebral cortex, cerebellum, and posterior pituitary, similar to the tissue distribution of endogenous mouse and rat vasopressin expression, and have normal water metabolism.

Habener *et al.* established a line of mice transgenic for mouse metallothionein I promoter-rat vasopressin structural gene[25]. They speculated that the sequence 5'GCCCAGCC3' between the TATA box and the cap site in the rat vasopressin gene is important in neuronal expression. The plasma vasopressin levels were approximately 100-fold higher than in control and serum osmolalities were also elevated and urine volume was elevated above normal, these results consistent with a state of nephrogenic diabetes insipidus. They postulated that there would be renal resistance from long-term exposure to high level of vasopressin in the transgenic mice.

Transgenic rats in our study:

Recently, we have generated rat transgenic for the fusion gene consisting of the heavy metal-inducible promoter region of the mouse metallothionein I gene and human vasopressin gene[26]. In this study, we aimed to show that 1) the heavy metal inducible metallothionein promoters may drive the vasopressin transgene, and 2) the transgene may be expressed not only in

nomotopic but also in ectopic tissues. Therefore, we placed the mouse metallothionein I promoter region 30 bp upstream of the translation initiation site of the human vasopressin structural gene. Although human vasopressin gene does not contain the octameric sequences locating in 5' UTR of rat vasopressin gene, we deleted most part of 5' and 3' UTR in transgene construction. However, in the transgenic rats we produced, higher expression of immunoreactive vasopressin was found in brain, and the distribution of immunoreactive vasopressin did not coincide well with the pattern predicted of a metallothionein-regulated gene. It might be possible that DNA elements located outside of the 5' and 3' UTR yet present within the vasopressin intron or coding regions may contribute to the cell-specific expression of vasopressin gene.

We intended to produce a line of transgenic rats that overexpress vasopressin gene to investigate the physiological alterations resulting from sustained hypervasopressinemia. We used rats as the host animal, because it would make physiological experiments much easier. We constructed a fusion gene consisting of 0.77 kb of the heavy metal-inducible promoter region in the mouse metallothionein I gene[27] and 2.1 kb of the human vasopressin structural gene (m-MT/h-VP), and a line of rats has been developed with transgene of m-MT/h-VP. Transgenic rat showed significantly higher immunoreactive vasopressin levels in the brain, heart, liver, spleen, pancreas, and testis. Among these tissues, the brain showed 100~500-fold higher levels than other tissues. Vasopressin mRNA was identified ubiquitously in the transgenic rat tissues, which corresponds to the wide distribution of immunoreactive vasopressin. To examine the authenticity of vasopressin in transgenic rats, we have analyzed the processing of provasopressin by gel filtration and bioactivity in LLC-PK1 cells. Efficient processing was identified in the brain, and less efficient processing in the pancreas and liver. Most immunoreactive vasopressin in the plasma proved to be properly-processed vasopressin. Therefore it was considered that serum vasopressin was processed properly and have authentic bioactivity.

In our study, plasma vasopressin levels in transgenic rat were 5 to 10 times higher than in control rats at basal conditions. We have revealed that in the rat, 1) plasma vasopressin immunoreactivity was remarkably elevated and increased further by administering zinc sulfate, 2) vasopressin was processed correctly and indicated almost equivalent bioactivity with authentic vasopressin, and 3) plasma sodium concentration was not different from control rats under basal condition, while it declined after water loading. Interestingly, the change in water and electrolyte balance was relatively mild in spite of a markedly elevated plasma vasopressin level. Considering with previous studies of vasopressin-overexpressing animals, our data suggest there are some adaptive mechanisms to keep plasma sodium levels in the chronically high plasma vasopressin.

Downregulation mechanisms in our transgenic rat:

Since our vasopressin overexpressing transgenic rat is continuously exposed to high plasma vasopressin, we postulate that there is a long-term adaptive response including down-regulation of V2R in kidney[28]. Although plasma vasopressin was markedly elevated, urine volume did not show dramatic changes and an almost equivalent AQP2 protein level in kidney was seen. Zinc liquid diet demonstrated disruption of renal water excretion and developed hyponatremia, however, the alteration in water and electrolyte balance was relatively mild. Renal reactivity to exogenously administered vasopressin clearly showed desensitization of its antidiuretic action.

There were several vasopressin overexpressing transgenic animals as mentioned above. However, no animal model demonstrated hyponatremia or water retention by vasopressin-induced antidiuresis. In our transgenic rat, urine volume slightly decreased with a normal plasma sodium

level and AQP2 protein amount in kidney under basal condition. Regarding the discrepancy of the results for urine volume and AQP2 protein, the distribution of AQP2 protein in the collecting duct cell might be altered in transgenic rat.

We investigated the effect of water loading and stimulation of vasopressin production from transgene by providing liquid diet containing zinc. Plasma vasopressin concentration potently increased in transgenic rat as expected but did not decrease in control rats unexpectedly. After liquid diet with zinc, water loading-induced diuresis was slightly suppressed in transgenic rat compared to control rats and plasma sodium decreased gradually and plateaued. In the overall, we confirmed physiological action of vasopressin in transgenic rat.

Verbalis *et al.* reported that continuous vasopressin infusion with excessive water administration results in water retention and hyponatremia[29]. However, urine volume increased in spite of sustained vasopressin infusion several days, this phenomenon is called the "vasopressin escape"[30,31]. In our transgenic animal, the escape from vasopressin action was already observed on the first day of water loading. Furthermore, hyponatremia was mild even when water loading was performed in rats with a plasma vasopressin level comparable to their study. These evidences indicate that a potent adaptive mechanism for maintaining water and electrolyte homeostasis might be established in the transgenic rat.

The antidiuretic effect of vasopressin is mediated by V2R, which belongs to a large family of G protein-coupled receptors (GPCRs) with a typical seven-transmembrane helix structure[32,33]. Continuous exposure to an agonist promotes desensitization of GPCRs through short- and long-term regulation. Long-term regulation often reflects changes in levels of mRNA or protein and/or changes in the turnover of receptor, down-regulation of receptor expression[34]. Receptor mRNA levels are frequently controlled by the degree of receptor stimulation. Receptor mRNA levels are therefore likely to be one of the most important control points for the regulation of receptor sensitivity.

Because we postulated that V2R down-regulation by long-standing high plasma vasopressin is responsible for the adaptive mechanism, we examined to reverse the down-regulation. Beta-adrenoceptor increases in number when beta-adrenoceptor blocking drugs are administered. Accordingly, we used nonpeptidic antagonist OPC31260[35], a benzazepine derivative, which does not induce visible receptor internalization[36]. Interestingly, the diuretic effect of OPC31260 by blocking V2R was attenuated in transgenic rat, in which V2R had been already blocked partly by the down-regulation. As expected, withdrawal of V2 antagonist pretreatment followed by water loading resulted in potentiation of vasopressin-induced antidiuresis in transgenic rat. Blood pressure was not altered by the zinc liquid diet or V2 antagonist in both transgenic rat and control rats, indicating that this effect is not due to some cardiovascular change. Northern blotting analysis for V2R demonstrated suppressed V2R mRNA expression under basal condition and its recovery after V2 antagonist treatment, was consistent with our hypothesis.

However, compared to the previous report of our laboratory regarding V2R mRNA down-regulation using the dehydrated normal rats[37], the suppression of V2R mRNA under the basal state of transgenic rat was less than the suppression in the dehydrated Sprague-Dawley rats, despite a higher plasma vasopressin level and much longer duration in transgenic rat than in the dehydrated normal rat of the previous study.

Although vasopressin has been reported to play an important role in cardiovascular regulation via vasopressin V1a receptors (V1aR), its precise significance remains unclear. We also investigated the effects of long-standing high plasma vasopressin level on cardiovascular regulation[38]. There were no significant differences in mean arterial blood pressure or heart rate between transgenic rats and control in the basal state. Exogenous injection of vasopressin significantly increased mean arterial blood pressure in control, but did not cause any apparent increase in

transgenic rats. Blood pressure recovery from hemorrhage-induced hypotension was significantly delayed in transgenic rats. To attenuate the downregulation of V1aR, pretreatment with a selective V1aR antagonist, OPC-21268, which restores the downregulation of V1aR, markedly improved both of these impaired responses. Northern blot analysis confirmed decreased expression of V1aR mRNA and that pretreatment with V1aR antagonist significantly restored the downregulation of V1aR mRNA. These results suggest that transgenic rats have decreased sensitivity to the vasoactive effect of vasopressin due to downregulation of V1aR. In addition, impaired restoration of bllod pressure after hemorrhage-induced hypotension in transgenic rat supports a physiological role of vasopressin in cardiovascular regulation.

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