Altered cardiovascular regulation in arginine vasopressin-overexpressing transgenic rat

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Tachikawa, Kazushige, Hisashi Yokoi, Hiroshi Nagasaki, Hiroshi Arima, Takashi Murase, Yoshisasa Sugimura, Yoshitaka Miura, Masumi Hirabayashi, and Yutaka Oiso. Altered cardiovascular regulation in arginine vasopressin-overexpressing transgenic rat. Am J Physiol Endocrinol Metab 285: E1161–E1166, 2003. First published August 12, 2003; 10.1152/ajpendo.00570.2002.—Although arginine vasopressin (AVP), an antidiuretic hormone, has been widely acknowledged to play an important role in cardiovascular regulation via V1a receptors (V1aR), its precise significance remains unclear. In this study, we investigated the effects of long-standing high plasma AVP status on cardiovascular regulation in the AVP-overexpressing transgenic (Tg) rat. Adult male homozygous Tg rats were compared with age-matched normal Sprague-Dawley rats as controls. There were no significant differences in mean arterial blood pressure (BP; MABP) or heart rate between Tg and control rats in the basal state. Subcutaneous injection of AVP significantly increased MABP in controls but did not cause any apparent increase in MABP in Tg rats. BP recovery from hemorrhage-induced hypotension was significantly delayed in Tg compared with control rats. Pretreatment with a selective V1aR antagonist, OPC-21268, which is thought to restore the downregulation of V1aR, markedly improved both of these impaired responses. Northern blot analysis confirmed that decreased expression of V1aR mRNA and pretreatment with V1aR antagonist significantly restored the downregulation of V1aR mRNA. These results suggest that the Tg rat has decreased sensitivity to the hypertensive effect of AVP due to downregulation of V1aR, which may function as an adaptive mechanism to maintain normal BP against chronic hypertensive. In addition, impaired restoration of BP after hemorrhage-induced hypotension in Tg rats supports a physiological role of AVP in cardiovascular regulation.

arginine vasopressin (AVP), an antidiuretic hormone, is well known to play an important role in cardiovascular regulation (1, 3, 6, 20). This hormone exerts potent vasoconstrictor action through V1a receptors (V1aR) in vascular smooth muscles (23, 27). It has been reported that a marked increase in plasma AVP following hemorrhage helps the recovery of blood pressure from hemorrhage-induced hypotension (11, 19, 26). Considerable evidence suggests the primary pathogenic role of AVP in some models of hypertension, such as the deoxycorticosterone (DOCA)-salt model (2, 8, 9, 21). Brattleboro rats with a hereditary AVP deficiency failed to develop hypertension when treated with DOCA-salt unless supplemented with AVP (5). Although the mechanism responsible for this is not fully understood, it has been considered that AVP may participate in the pathogenesis of DOCA-salt hypertension by increasing sympathetic outflow in addition to its peripheral vasoconstrictor and renal actions (4). However, it is generally considered that chronic elevation of plasma AVP does not produce sustained hypertension (6, 7, 17). Transient volume expansion and sustained hyponatremia with no hypertension are the most prominent features observed in chronic elevations of plasma AVP in dogs and humans (13, 22). These findings suggest that there may be some adaptive mechanism against long-term AVP elevation to maintain normal cardiovascular regulation. However, the precise mechanism responsible for this remains unclear.

Recently, we (15, 25) developed rats transgenic for the fusion gene consisting of the heavy metal-inducible promoter region of the mouse metallothionein I gene and human AVP gene (Tg). Interestingly, the change in water and electrolyte balance was relatively mild despite a markedly elevated plasma AVP level, suggesting that there may be adaptive mechanisms for maintaining water and electrolyte homeostasis against chronic AVP oversecretion from the earliest stage of life.

In the present study, to elucidate the physiological importance of AVP in the cardiovascular regulation, we examined the effect of chronic hypervasopressinemia on cardiovascular changes by use of our Tg rats. We evaluated the vascular responsiveness of Tg rats to exogenous AVP, as well as to endogenous AVP that is stimulated by hemorrhage-induced hypotension. We also attempted to restore the downregulation of V1aR by V1aR antagonist pretreatment and reexamined the blood pressure responsiveness to AVP.

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MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats homozygous for the transgene (Tg) and age-matched controls (12–16 wk old; Chubu Science Materials, Nagoya, Japan) were used. Details of the preparation of the rat transgenic for the human AVP gene used in this study have been previously reported (15). Rats were maintained under controlled conditions (23.0°C; lights on from 0900 to 2100), and all procedures were performed in accordance with institutional guidelines for animal care at Nagoya University School of Medicine, which conform to the National Institutes of Health animal care guidelines.

Materials. A diet containing a V1αR antagonist, the orally effective, nonpeptidic, selective V1αR antagonist OPC-21268 (a gift from Otsuka Pharmaceutical, Tokyo, Japan) was mixed with standard rat chow at a concentration of 0.05% (24).

Experimental design of V1αR pretreatment. In our previous study (25), we successfully restored the downregulation of V2 receptors (V2R) by pretreatment with a V2R antagonist. In the present study, we attempted to restore the downregulation of V1αR by pretreatment with the V1αR antagonist OPC-21268. Control and Tg rats were divided into two groups: without V1αR antagonist and with V1αR antagonist. In the group without V1αR antagonist, rats were fed standard rat chow on all days. A diet containing AVP V1αR antagonist OPC-21268 (0.05%) was provided for 4 days, and rats were then switched to the standard rat chow in the group with V1αR antagonist. In our preliminary experiments, subcutaneous injection of AVP did not increase blood pressure on day 1 after rats were switched to the standard diet, whereas it markedly increased it on day 2 (data not shown). These results indicated that, although the V1αR antagonistic effect of OPC-21268 remains on day 1 even after the switch to the standard diet, it is no longer effective on day 2. Therefore, the following experiments to evaluate the effect of exogenous or endogenous AVP on blood pressure were performed on day 2 after the switch (Fig. 1).

Surgical procedures. Two days before the experiment, the rats were anesthetized with pentobarbital sodium (50 mg/kg body wt) by intraperitoneal injection, and a PE-50 polyethylene catheter, pulled to a fine tip, was implanted in the right carotid artery. The catheter was filled with heparinized (100 U/ml) 0.9% saline and then sealed and exteriorized at the nape of the neck. Each rat was housed in an individual cage in which it was allowed to move freely.

Experiment 1: effects of subcutaneous injection of synthetic AVP on blood pressure. Rats were injected subcutaneously with synthetic AVP (0.6 μg/kg body wt; Peptide Institute, Osaka, Japan). Arterial blood pressure was continuously recorded before and after experimental manipulation by connecting the arterial line to a blood pressure transducer (Soft Run BP-98A, OMRON, Tokyo, Japan) via an amplifier connected to a hot-wire recorder to measure pulsatile blood pressure. Mean arterial blood pressure (MABP) was determined as the diastolic pressure plus one-third of the pulse pressure.

Experiment 2: effects of hemorrhage on arterial blood pressure. Hemorrhage was accomplished by withdrawing 16 ml/kg body wt of total blood volume via blood removal over a 3-min period from the right carotid cannula. It has been estimated that 8 ml/kg body wt is ~10% of the total blood volume (12). Arterial blood pressure was continuously recorded with a blood pressure transducer connecting the arterial line implanted into the right carotid artery.

Preparation of RNA and Northern blotting. Rats were fed a diet containing V1αR antagonist for 4 days or fed continually with standard chow. They were killed by decapitation, and the liver was rapidly removed and frozen at −80°C. Total RNA was extracted from the liver using TRIzol reagent (Life Technologies, Rockville, MD) according to the manufacturer’s recommendation. The amount of RNA was determined by the spectrophotometric method. Each 10 μg of total RNA was size-fractionated and transferred to a nylon membrane (Gene Screen Plus Hybridization Membrane; Biotechnology Systems NEN Research Products, Boston, MA) for Northern blot hybridization by use of a 32P-labeled 602-base pair cDNA probe designed specifically for rat V1α R DNA. The probe was generated by polymerase chain reaction (10). To ensure even loading, the membrane was washed and then rehybridized with a rat GAPDH cDNA probe. The integrated radioactivity of the mRNA band for V1αR and GAPDH was quantified with a Bio-Image Analyzer (BAS2000; Fuji Photo Film, Tokyo, Japan). The changes in V1αR mRNA expression are presented as the percentage of changes from nontreated control rats after correction for GAPDH mRNA expression.

Plasma AVP measurement. Blood samples were obtained via the carotid cannula before and 10 min after hemorrhage (16 ml/kg) and collected in chilled tubes containing EDTA (potassium salt) for AVP assay. After immediate separation, plasma AVP was extracted through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) and measured using a highly sensitive radioimmunoassay kit (AVP-RIA kit, kindly provided from Mitsubishi Chemical, Tokyo, Japan). The changes in V1αR mRNA expression are presented as the percentage of changes from nontreated control rats after correction for GAPDH mRNA expression.

Fig. 1. Experimental protocol of V1α receptor (V1αR) antagonist pretreatment. A: without V1αR antagonist. Rats were fed standard rat chow on all days. B: with V1αR antagonist. A diet containing arginine vasopressin (AVP) V1αR antagonist OPC-21268 (0.05%) was provided for 4 days, and then animals were switched to standard rat chow. A cannula was implanted in the right carotid artery for blood pressure (BP) measurement on day 1. Experiments were performed on day 2.
RESULTS

**Basal measurements.** The plasma AVP level was markedly elevated in Tg rats. Under basal condition, there were no significant differences in MABP and heart rate between controls and Tg rats (Table 1).

**Experiment 1: effects of subcutaneous injection of AVP on blood pressure.** In rats without V1aR antagonist pretreatment, subcutaneous injection of AVP significantly increased MABP in controls, whereas there was no rise of MABP after AVP injection in Tg rats. In rats with V1aR antagonist pretreatment, however, the AVP injection significantly increased MABP in Tg rats to the same level as in controls (Fig. 2).

**Experiment 2: effects of hemorrhage on arterial blood pressure.** The hemorrhage caused a marked decrease in MABP in both control and Tg rats with or without V1aR antagonist pretreatment. In control rats, within 20 min, MABP quickly recovered to the same levels as before hemorrhage. On the other hand, in Tg animals without V1aR antagonist pretreatment, this MABP recovery after hemorrhage was significantly delayed compared with controls. However, in Tg rats pretreated with V1aR antagonist, MABP after hemorrhage-induced hypotension quickly recovered within 20 min, the same as in the controls (Fig. 3). The plasma

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**Table 1. Basal measurements**

<table>
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<tr>
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<th>n</th>
<th>Body Weight, g</th>
<th>MABP, mmHg</th>
<th>HR, beats/min</th>
<th>Plasma AVP, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>388.8 ± 10.8</td>
<td>106.4 ± 4.8</td>
<td>422 ± 18</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Tg</td>
<td>7</td>
<td>345.5 ± 13.0*</td>
<td>109.9 ± 4.9</td>
<td>435 ± 64</td>
<td>13.8 ± 2.8*</td>
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Values are means ± SE; n, no. of rats. MABP, mean arterial blood pressure; HR, heart rate; AVP, arginine vasopressin. Tg, AVP-overexpressing transgenic. *P < 0.05 vs. controls.

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**Fig. 2.** Effects of AVP on mean arterial blood pressure (MABP) without (A) and with V1aR antagonist (B). AVP (0.6 μg/kg body wt) was injected sc in control (○) and AVP-overexpressing transgenic (Tg) (●) rats; n = 3 for Tg rats in A, n = 4 for the other 3 groups. *P < 0.05 vs. controls; †P < 0.05 vs. 0 min.

**Fig. 3.** Effects of hemorrhage on MABP. A: without V1aR antagonist. B: with V1aR antagonist. Hemorrhage (16 ml/kg) was accomplished via blood removal from the right carotid cannula in control (○) and Tg (●) rats; n = 4 for each group. *P < 0.05 vs. control.
AVP levels after the hemorrhage were higher in Tg compared with those of the control rats both without and with V1aR antagonist. There was no significant difference in plasma AVP levels after hemorrhage between Tg rats with and without V1aR antagonist (Fig. 4).

**V1aR mRNA expression.** To confirm downregulation of V1aR in Tg rats, we first performed Northern blotting with samples from the aorta to examine the V1aR expression in vascular tissues. However, we could not detect any apparent bands, possibly because the amount of sample obtained from each animal was too small (data not shown). Instead, we selected the liver to evaluate the V1aR expression changes, because it shows the highest expression of V1aR after vascular tissues (10, 14). In Tg rats, V1aR mRNA expression in the liver was significantly less than in control rats under the basal condition, and this suppression was restored by V1aR antagonist pretreatment to levels comparable with those of control rats. V1aR antagonist treatment had no significant effect on V1aR mRNA expression in the liver of control rats (Fig. 5).

**DISCUSSION**

We (15) recently established AVP-overexpressing Tg rats to investigate the effects of sustained high plasma AVP levels on various homeostatic changes. In the previous study, we examined water and electrolyte metabolism in Tg rats, which is regulated by AVP mainly via V2R in kidney, and showed that there was an adaptive mechanism to prevent water retention caused by sustained hypervasopressinemia (25). Another major action of AVP is vasoconstriction, which is mediated by V1aR in vascular smooth muscle cells. Therefore, in the present study, we evaluated the alteration of cardiovascular regulation in Tg rats. First, we compared blood pressure at the basal state between Tg and control rats. Because AVP has a strong vasoconstrictive action, it may be possible that Tg rats would show elevated blood pressure levels. Interestingly, however, Tg rats showed no significant differences in basal MABP and heart rate compared with controls despite sustained high plasma AVP levels, suggesting that Tg rats have a lower sensitivity to the hypertensive effect of AVP.

Next, we examined the effect of exogenously administered AVP on blood pressure in Tg rats. As a result, the blood pressure increase after AVP injection was significantly attenuated in Tg compared with control rats, indicating that Tg rats are resistant to the hypertensive effect of AVP. This finding is similar to our previous report about the antidiuretic effects of AVP. Despite high plasma AVP levels, Tg rats showed almost the same urine volume and water intake as controls because of decreased renal sensitivity to the antidiuretic effect of AVP. We found that this attenuation...
was caused mainly by downregulation of kidney V2R (25). It is reasonable to think that a similar mechanism may explain the decreased sensitivity to the hypertensive effect of AVP. Unlike a peptide antagonist, the nonpeptide V2R antagonist OPC-31260 has been reported not to induce downregulation of V2R (18). Furthermore, we showed in the previous study that V2R antagonist restored downregulation of kidney V2R in Tg rats and that V2R antagonist pretreatment resulted in potentiation of the antidiuretic effect of AVP (25).

Thus we gave rats V1aR antagonist to restore downregulation of kidney V2R. We then reexamined the effect of exogenously injected AVP on blood pressure. Results showed that, after pretreatment with V1aR antagonist, the hypertensive effect of AVP was significantly increased in Tg rats to the same level as that of controls.

To confirm downregulation of V1aR in Tg rats, we performed Northern blotting with samples from the liver to examine the V1aR expression changes. V1aR mRNA expression in the liver proved to be significantly decreased in Tg compared with control animals and was reincreased to the same level as that of controls after V1aR antagonist pretreatment. Thus downregulation of V1aR in the liver was restored by V1aR antagonist pretreatment. Although we did not show directly the V1aR expression in vascular smooth muscle, it seems quite possible that similar changes occurred there.

Next, we examined the responsiveness of Tg rats to endogenous AVP in the condition in which endogenous AVP is markedly stimulated. The blood pressure recovery from hemorrhage-induced hypotension was significantly delayed in Tg compared with control rats. The plasma AVP level after hemorrhage was higher in Tg compared with control rats. Therefore, endogenous AVP release induced by hemorrhage was not impaired despite sustained high plasma AVP levels, and it is unlikely that a delay in blood pressure recovery in Tg rats was due to an impaired response of AVP release to hypotension. Moreover, blood pressure recovery from hemorrhage-induced hypotension was markedly improved after restoration of V1aR downregulation by pretreatment with the V1aR antagonist. There were no significant differences in plasma AVP levels after hemorrhage between Tg rats with and without V1aR antagonist, indicating that the improvement of blood pressure recovery by V1aR antagonist is not due to the changes in plasma AVP levels by V1aR antagonist. These results suggest that there was a delay in blood pressure recovery from hypotension in Tg rats because of decreased vascular sensitivity to the hypertensive effect of endogenous AVP due to the downregulation of V1a receptors. It is generally accepted that AVP plays an important role in raising blood pressure upon recovery from hemorrhage-induced hypotension via V1a receptors. It has been reported that V1aR antagonist significantly delays blood pressure recovery from hemorrhage (11, 19, 26).

In conclusion, the results of the present study suggest that the Tg rat has decreased sensitivity to the hypertensive effect of AVP due to downregulation of V1aR, which may function as an adaptive mechanism to maintain normal blood pressure against chronic hypervasopressinemia. In addition, the impaired restoration of blood pressure after hemorrhage-induced hypotension in the Tg rat supports the physiological role of AVP in cardiovascular regulation.

DISCLOSURES
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