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Cardiovascular Depression and Stabilization by Central Vasopressin in Rats

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The role of endogenous vasopressin in cardiovascular homeostasis was examined using vasopressin-deficient rats (Brattleboro) ($n=194$) and their parent strain, Long-Evans rats ($n=181$). Mean arterial pressure (blood pressure) and heart rate were measured every 4 seconds with or without infusion of drug solution for 21 hours, and mean values and their standard deviations (lability) were calculated. Blood pressure in Brattleboro rats (116 ± 1.1 mm Hg, mean \pm SEM) was significantly higher than that in Long-Evans rats (96 ± 0.7 mm Hg, $p<0.001$), whereas heart rates (381 ± 3.3 and 375 ± 2.9 beats/min, respectively) were similar. The lability of blood pressure and heart rate in Brattleboro rats (9.2 ± 0.1 mm Hg and 42.3 ± 0.7 beats/min) was also greater than that in Long-Evans rats (6.7 ± 0.1 mm Hg, $p<0.001$ and 38.4 ± 0.8 beats/min, $p<0.01$, respectively). In Brattleboro rats, intravenous vasopressin (0.1 ng/kg/min or 0.6 ng/kg/min) did not affect blood pressure, although it did reduce heart rate and decreased lability of blood pressure and heart rate. Intracerebroventricular (central) infusion of vasopressin (2 pg/kg/min) in Brattleboro rats induced initial hypertension and tachycardia followed by long-lasting hypotension and bradycardia, whereas in Long-Evans rats it induced only hypertension and tachycardia. In both strains, central vasopressin dramatically decreased the lability of blood pressure and heart rate. Neither intravenous (0.2 ng/kg/min) nor central desmopressin (2 pg/kg/min or 0.2 ng/kg/min), a V_2 renal receptor agonist, changed any of these parameters in Brattleboro rats, although both diminished urinary volume. Neither intravenous (50 ng/kg/min) nor central (3.3 pg/kg/min) $d(\text{CH}_2)_5\text{-Tyr(Me)-arginine}$ vasopressin, a vasopressin V_1 receptor antagonist, modulated any of these parameters in Long-Evans rats. These results suggest that endogenous as well as exogenous vasopressin acts centrally as a cardiovascular inhibitor and stabilizer through a receptor mechanism other than V_1 or V_2 receptor mechanisms. (*Hypertension* 1990;15:291-300)

Arginine vasopressin (AVP) has long been known to possess potent vasoconstrictor properties.¹ However, when AVP is infused intravenously into conscious animals or humans, there is no increase in blood pressure until extremely high plasma AVP levels are achieved.² Evidence has accumulated indicating that the absence of increases in blood pressure in response to AVP infusion in normotensive animals may be due to the presence of

an effective and specific buffering system.³⁻⁵ On the basis of available evidence, it has been proposed that the locus of this buffering system is in the central nervous system.⁶⁻¹² Recently, it has been reported that Brattleboro rats with hereditary hypothalamic diabetes insipidus (DI rats) have suppressed baroreceptor reflex sensitivity.⁹ Exogenous AVP and the V_2 renal receptor agonist desmopressin (DDAVP) administered systemically is able to restore this suppressed sensitivity to within the range that is normal for Long-Evans (LE) rats.⁹ It is believed that the inhibition of baroreceptor reflex pathway raises blood pressure.¹³ Therefore, it may be postulated that rats with suppressed baroreceptor reflex mechanisms will have higher blood pressure and heart rate and a greater lability of blood pressure and heart rate than normal rats. Furthermore, we previously reported that centrally administered AVP lowered blood pressure in DI rats as well as in stroke-prone

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spontaneously hypertensive rats (SHR), whereas it elevated blood pressure in normal LE rats and Wistar-Kyoto (WKY) rats.^{14,15} This line of evidence encouraged us to examine the difference in blood pressure and heart rate characteristics between DI and LE rats; in the former, AVP was lacking in the central nervous system as well as in the peripheral circulation. In the present study, we examined the effect of a small amount of AVP or vasopressin analogues intravenously or intracerebroventricularly infused over 21 hours on blood pressure, heart rate, and lability of blood pressure and heart rate in DI rats as well as in LE rats. To analyze the characteristics and responses of blood pressure and heart rate accurately, we used continuous blood pressure and heart rate monitoring systems in conscious, unrestrained rats and analyzed the data with a microcomputer.

Methods

Animals

DI rats and their parent strain, LE rats, weighing 150–400 g and 15–45 weeks of age, were used. These rats were divided into groups matched by age: 15–19-week-old group, LE rats 18.1 ± 0.6 weeks, 275 ± 13 g, mean \pm SD, $n=33$ and DI rats 18.0 ± 0.5 weeks, 252 ± 14 g, $n=32$; 20–24-week-old group, LE rats 22.9 ± 0.5 weeks, 298 ± 9 g, $n=45$, DI rats 23.5 ± 0.4 weeks, 270 ± 13 g, $n=40$; 25–29-week-old group, LE rats 27.6 ± 0.3 weeks, 348 ± 18 g, $n=34$, DI rats 27.3 ± 0.3 weeks, 312 ± 15 g, $n=32$; 30–35-week-old group, LE rats 33.0 ± 0.4 weeks, 369 ± 10 g, $n=35$, DI rats 32.0 ± 0.5 weeks, 340 ± 13 g, $n=55$; and 35-week-old group, LE rats 39.6 ± 1.4 weeks, 378 ± 14 g, $n=34$, DI rats 40.1 ± 0.8 weeks, 348 ± 19 g, $n=35$. The rats were placed into individual plastic metabolic cages and housed under conditions of constant temperature ($23 \pm 1^\circ\text{C}$) and humidity (60%), with a 12-hour light/dark cycle. The rats were housed in an isolated room, but the room was not soundproof. The rats were continuously exposed to the noise from infusion pumps and neighboring rats. The environmental conditions were common for all experiments. The rats were fed a commercial diet and water ad libitum.

Surgical Procedure and Measurement of Arterial Pressure and Heart Rate

While the rats were under ether anesthesia, the left femoral artery and vein were cannulated with polyethylene tubing. The tip of the arterial catheter (Intramedic PE-100; Clay Adams, Parsippany, New Jersey) was tapered by heating for insertion into the femoral artery. In some rats, venous catheters (Intramedic PE-20) were implanted bilaterally. The catheters were passed subcutaneously and brought out on the neck. During surgery, 15 mg aminobenzyl penicillin was administered topically and intraperitoneally. The arterial catheter was connected to a hydraulic swivel-tethering system. Rats were allowed to recover for at least 24 hours after surgery and were conscious and unrestrained during the subsequent

studies. Blood pressure was recorded from the femoral artery catheter with a P231D Statham pressure transducer (Oxnard, California) and strain-gauge amplifier (model 1257, NEC-San-ei, Tokyo, Japan). To keep the arterial catheter patent, heparin physiological saline solution (100 units/ml) was infused continuously at a rate of $80 \mu\text{l/hr}$. Heart rate was counted from the phasic pressure pulse by a cardi-tachometer (model 1321, NEC-San-ei). The analog signals of phasic pressure, mean arterial pressure, and heart rate were fed into an analog/digital (A/D) converter (Mark-1, N.C.C., Tokyo, Japan). Digital signals of mean arterial pressure and heart rate from the A/D converter were fed into a microcomputer (HP-9816, Hewlett-Packard, Fort Collins, Colorado) at 4-second intervals over a period of 21 hours. The recording was started at any time between 9:00 AM and 6:00 PM. At the end of the measurement period, the processed data was printed (Hewlett-Packard 82906A). Mean values and standard deviation for every hour, time trend-gram, mean values and standard deviation for 21 hours, and percent frequency histograms for each parameter were put out. Mean values and standard deviations of blood pressure and heart rate for every hour and for 21 hours were calculated by using all data sampled at 4-second intervals for each period. The lability of mean arterial pressure and heart rate was expressed as standard deviation of each parameter averaged over 21 hours or every hour. Analog data was monitored simultaneously with a recticorder (Rectigraph 8K, NEC-San-ei). While under pentobarbital anesthesia (40 mg/kg i.p., Nembutal, Abbott, North Chicago, Illinois), the lateral cerebroventricle in some LE and DI rats was cannulated (Intramedic PE-20, 4.6 cm in length with approximate capacity of $4.6 \mu\text{l}$). Coordinates for the cannulation with respect to the bregma were 1.0 mm posterior, 1.5 mm lateral, and 5.0 mm deep. The cannulae were filled with artificial cerebrospinal fluid (ACSF) (mmol/l) (sodium 148.4, potassium 3.0, magnesium 1.0, calcium 2.5, HPO_4 1.5, chloride 156.9, glucose 3) and fixed in place with stainless steel anchoring screws and orthopedic bone cement. A week after the cerebroventricular cannulation, the femoral artery and vein were cannulated.

Experimental Protocol

In the following study 25–29-week-old rats were used. In all experiments, a time control base was obtained with either intracerebroventricular ACSF infusion at a rate of $4.6 \mu\text{l/hr}$ or intravenous infusion of physiological saline solution at a rate of $40 \mu\text{l/hr}$.

Intravenous infusion studies. Mean arterial pressure and heart rate in DI rats were measured with and without intravenous infusion of either AVP (Protein Research Foundation, Osaka, Japan) (at a rate of 2 pg/kg/min for 21 hours [$n=7$], 0.1 ng/kg/min for 21 hours [$n=8$], or 0.6 ng/kg/min for 21 hours [$n=7$]) or 1-(3-mercaptopropionic acid)-8-D-AVP (desmopressin) (DDAVP, Ferring, Malmo, Sweden), a V_2 renal receptor agonist (0.2 ng/kg/min for 21 hours

[$n=7$]). In LE rats, mean arterial pressure and heart rate were measured with and without intravenous infusion of 1-(β -mercapto- β , β -cyclopentamethylene-propionic acid),2-(O-methyl) tyrosine-AVP, [$d(\text{CH}_2)_5$ -Tyr(Me)]-AVP (Peninsula, San Carlos, California¹⁶), a V_1 vascular receptor antagonist (AVP antagonist) at a rate of 50 ng/kg/min for 21 hours ($n=7$).

Intracerebroventricular infusion studies. In 21 DI rats, blood pressure and heart rate were measured for 21 hours during intracerebroventricular infusion of ACSF on the third and fourth day after surgery, and the reproducibility of blood pressure, heart rate, and their lability were evaluated.

AVP (2 pg/kg/min in eight LE rats and 13 DI rats), desmopressin (2 pg/kg/min and 0.2 ng/kg/min in each of six DI rats), or AVP antagonist (3.3 pg/kg/min in eight LE rats) was infused intracerebroventricularly for 21 hours, and blood pressure and heart rate (mean and standard deviation) were examined.

All stock solutions (dissolved in 0.1N acetic acid) were diluted with 0.9% saline or ACSF to the desired concentrations. The venous and cerebroventricular catheters were connected to PE-20 tubing, which was attached to a Princeton infusion pump (model 575, Natick, Massachusetts). Drug solutions were infused intravenously at a rate of 40 μ l/hr and intracerebroventricularly at a rate of 4.6 μ l/hr. Intravenous and central drug infusion lasted 21 hours, the initial hour of which was required to clear the dead space of the venous or cerebroventricular cannula. The data of the initial hour of intracerebroventricular drug infusion were estimated as the control for time sequence analysis and expressed as preinfusion period in the figure (0 hour). In all experiments, 21-hour urine samples were collected.

Statistical Analysis

All values were expressed as mean \pm SEM unless otherwise stated. Statistical tests included one-way analysis of variance (ANOVA), two-way ANOVA for repeated measurements, and Duncan's multiple range test where appropriate.

Results

Blood Pressure, Heart Rate, and Their Lability in Long-Evans and Diabetes Insipidus Rats

The mean arterial pressure and its standard deviation (lability) in DI rats averaged for the 21-hour study period (116 \pm 1.1 mm Hg and 9.2 \pm 0.1 mm Hg, respectively, $n=194$) were significantly higher than those in LE rats (96 \pm 0.7 mm Hg, $F=21.3$ and 6.7 \pm 0.1 mm Hg, $F=13.9$, respectively, $n=181$, $p<0.001$). The lability of heart rate, averaged for the 21-hour study period in DI rats (42.3 \pm 0.7 beats/min), was also greater than that in LE rats (38.4 \pm 0.8 beats/min, $F=7.2$, $p<0.01$); the heart rate averaged for the 21-hour study period in DI rats (381 \pm 3.3 beats/min) was similar to that in LE rats (375 \pm 2.9 beats/min, $F=3.7$, $p>0.05$). Figure 1 presents typical records of mean arterial pressure for the 21-hour

study period in LE and DI rats, respectively. Figure 2 demonstrates the relation between age (weeks) and each parameter. The mean arterial pressure and its standard deviation, averaged for the 21-hour study period in DI rats, were significantly higher than those in LE rats of each age group. The heart rate, however, was similar in both groups of rats at all ages. The standard deviation of the heart rate, averaged for the 21-hour study period in DI rats younger than 30 weeks, was higher than that in LE rats of the same age. This difference was not evident in rats over 30 weeks of age. The lability of blood pressure and heart rate in DI rats as demonstrated by variation coefficient was still significantly higher in DI rats than that in LE rats (Table 1).

In the following experiments, 25–29-week-old rats were used. Table 2 shows basal values and standard deviations of blood pressure and heart rate averaged for 1 hour in the control period (0 hour).

Effects of Intravenously Administered Arginine Vasopressin, Desmopressin, or Arginine Vasopressin Antagonist on Blood Pressure, Heart Rate, and Their Lability

Figure 3A demonstrates the effect of intravenous AVP (0.6 ng/kg/min for 21 hours) in DI rats ($n=7$) on each variable. AVP did not affect the mean arterial pressure averaged for the 21-hour study period (0.1 ng/kg/min: 117 \pm 0.8 vs. 115 \pm 0.9 mm Hg; 0.6 ng/kg/min: 106 \pm 1.0 vs. 107 \pm 2.3 mm Hg); whereas it did induce a significant bradycardia (0.1 ng/kg/min: 372 \pm 9.5 vs. 360 \pm 3.9 beats/min; 0.6 ng/kg/min: 369 \pm 8.7 vs. 349 \pm 4.9 beats/min, $p<0.01$, respectively) and did decrease the standard deviations of mean arterial pressure (0.1 ng/kg/min: 9.2 \pm 0.7 vs. 7.2 \pm 0.3 mm Hg; 0.6 ng/kg/min: 8.5 \pm 0.5 vs. 6.9 \pm 0.4 mm Hg, $p<0.01$, respectively) and heart rate (0.1 ng/kg/min: 43.3 \pm 1.9 vs. 39.6 \pm 1.2 beats/min; 0.6 ng/kg/min: 44.1 \pm 2.1 vs. 38.0 \pm 2.6 beats/min, $p<0.01$, respectively) averaged for the 21-hour study period, suggesting the stabilization of mean arterial pressure as well as heart rate. Figure 4 represents the results of time–sequence analysis of blood pressure, heart rate, and their lability (standard deviations) with and without intravenous infusion of AVP at a rate of 0.6 ng/kg/min. It is apparent that heart rate (two-way ANOVA, $F_{1,272}=36.1$, $p<0.0001$) and lability of mean arterial pressure ($F_{1,272}=38.1$, $p<0.0001$) and heart rate ($F_{1,272}=21.2$, $p<0.0001$) dramatically decreased immediately after the start of infusion. These doses of AVP significantly decreased the 21-hour urinary volume (0.1 ng/kg/min: 303 \pm 52 to 175 \pm 12 ml; 0.6 ng/kg/min: 263 \pm 31 to 119 \pm 9 ml, $p<0.001$, respectively), although this was still above the normal range in LE rats. AVP infused intravenously at a rate of 2 pg/kg/min did not affect blood pressure, heart rate, or their lability.

Figure 3B illustrates the effect of intravenous desmopressin (0.2 ng/kg/min for 21 hours) in DI rats ($n=7$) on each variable. This dose reduced the production of urine from 339 \pm 42 to 18 \pm 3 ml/21 hours. However,

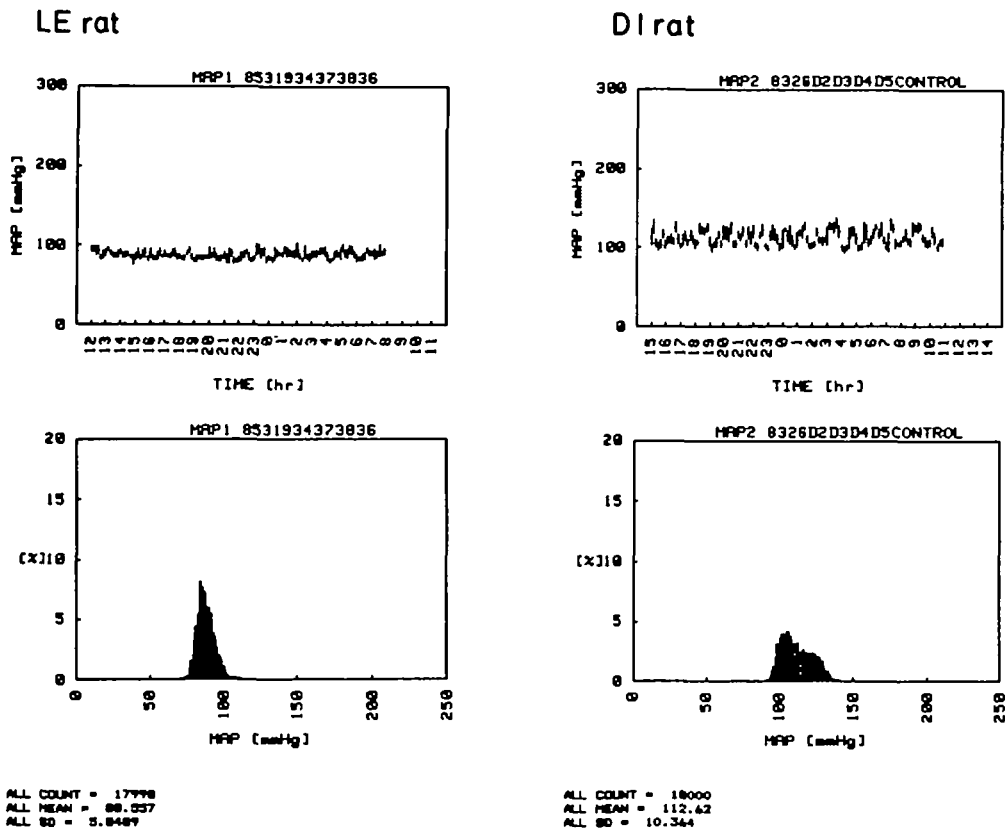


FIGURE 1. Upper panels present a typical recording of the time trend of mean arterial pressure (MAP) over 21-hour study period in Long-Evans (LE) rat (left side panel) and in Brattleboro (DI) rat (right side panel). Each point represents mean \pm SD of measurements over each 10-minute period. Bottom panels present percent frequency histogram of MAP values over 21-hour period.

desmopressin did not affect mean arterial pressure (127 ± 2.6 vs. 131 ± 2.8 mm Hg), heart rate (364 ± 10.6 vs. 361 ± 14.6 beats/min), and the standard deviations of mean arterial pressure (10.3 ± 0.6 vs. 10.9 ± 0.8 mm Hg)

and heart rate (42.1 ± 2.6 vs. 39.6 ± 1.3 beats/min) averaged for the 21-hour study period.

As shown in Figure 3C, intravenous AVP antagonist (50 ng/kg/min for 21 hours) did not cause any

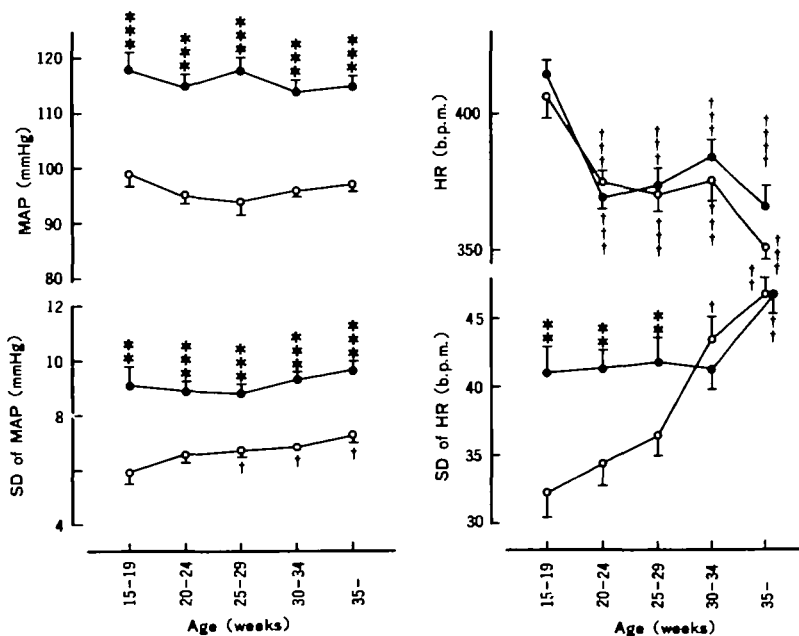


FIGURE 2. Line graphs showing mean arterial pressure (MAP), heart rate (HR), and standard deviations (SD) of MAP and HR (lability) averaged for 21-hour period in Long-Evans (LE) (○) and Brattleboro (●) rats of various ages. **p<0.01; ***p<0.001 compared with normal LE rats. †p<0.05; ††p<0.01; †††p<0.001 compared with 15-19-week-old rats (Duncan's multiple range test).

TABLE 1. Variation Coefficient of Mean Arterial Pressure and Heart Rate in Age-Matched Long-Evans and Brattleboro Rats

Rats		Age (wk)				
		15–19	20–24	25–29	30–34	35+
LE	MAP	0.059±0.005	0.069±0.002	0.071±0.003	0.071±0.003	0.075±0.003
	HR	0.079±0.007	0.092±0.004	0.099±0.004	0.116±0.008	0.133±0.004
DI	MAP	0.077±0.005*	0.078±0.003†	0.075±0.003	0.082±0.003*	0.084±0.004†
	HR	0.099±0.006†	0.111±0.005*	0.112±0.005*	0.107±0.004	0.128±0.006

Values are mean±SEM. LE, Long-Evans rats; MAP, mean arterial pressure; HR, heart rate; DI, Brattleboro rats. † $p < 0.05$; * $p < 0.01$ compared with Long-Evans rats.

significant change in mean arterial pressure (101 ± 2.9 vs. 100 ± 2.9 mm Hg), heart rate (335 ± 7.2 vs. 333 ± 6.9 beats/min), and the standard deviations of mean arterial pressure (7.6 ± 0.5 vs. 8.3 ± 0.5 mm Hg) and heart rate (41.1 ± 3.4 vs. 38.6 ± 3.2 beats/min) averaged for the 21-hour study period. Thirty minutes after cessation of the AVP antagonist infusion, the pressor effect of AVP in a dose of 100 ng/kg (51.7 ± 1.8 mm Hg before AVP antagonist infusion) was completely inhibited.

Effects of Centrally Administered Arginine Vasopressin, Desmopressin, or Arginine Vasopressin Antagonist on Blood Pressure, Heart Rate, and Their Lability

Intracerebroventricular infusion of ACSF did not cause any significant change in blood pressure, heart rate, or their lability throughout the infusion period, and the time-dependent variations in each parameter were reproducible between the two experiments of ACSF infusion.

Because intracerebroventricular infusion of AVP or AVP analogue induced biphasic or triphasic responses, a time–sequence analysis was performed.

Intracerebroventricular infusion of AVP to LE rats caused a gradual increase in mean arterial pressure that reached a peak 4 hours after the start of the infusion. The mean arterial pressure remained above the control level (0 hour) during the 20th hour of the infusion (Figure 5A, two-way ANOVA, $F_{1,314} = 72.5$,

$p < 0.0001$). In DI rats, an initial rise in mean arterial pressure that lasted approximately 8 hours (0–9 hour, $F_{1,249} = 14.6$, $p < 0.0002$) was followed by long-lasting hypotension (Figure 5B) (10–20 hour, $F_{1,274} = 23.9$, $p < 0.0001$). The lability of blood pressure (standard deviation of blood pressure averaged every hour) in both strains decreased immediately after the start of the AVP infusion (Figure 5). This stabilizing effect on the blood pressure lasted throughout the infusion in DI rats ($F_{1,503} = 40.7$, $p < 0.0001$), whereas lability of blood pressure tended to recover to its control level in LE rats ($F_{1,314} = 15.6$, $p < 0.0001$). Intracerebroventricular AVP caused a transient increase in heart rate in both strains. In LE rats, heart rate returned to the control level 4–8 hours after the start of the infusion and then increased again and persisted throughout the remainder of the infusion (Figure 6A) ($F_{1,314} = 28.8$, $p < 0.0001$). On the other hand, in DI rats an initial tachycardia (statistically not significant; 0–5 hour, $F_{1,149} = 3.46$, $p > 0.05$) was followed by a long-lasting bradycardia (Figure 6B) (6–20 hour, $F_{1,374} = 151.6$, $p < 0.0001$). Urinary volume was not affected by intracerebroventricular infusion of AVP in DI rats (315 ± 29.1 vs. 302 ± 32.5 ml/21 hours). The lability of heart rate in both strains decreased immediately after the start of the AVP infusion (LE rats $F_{1,314} = 11.2$, $p < 0.001$; DI rats $F_{1,503} = 15.9$, $p < 0.0001$). In both strains, the lability of heart rate recovered to its control level as the infusion continued.

TABLE 2. Control Values of Blood Pressure, Heart Rate, and Lability of Blood Pressure and Heart Rate

Experiment	n	MAP (mm Hg)	HR (beats/min)	SD of MAP (mm Hg)	SD of HR (beats/min)
DI i.v. AVP (2 pg/kg/min)	7	113±7.0	354±8.6	8.3±0.9	39.4±3.7
DI i.v. AVP (0.1 ng/kg/min)	8	115±6.9	360±10.3	7.4±0.8	38.2±3.9
DI i.v. AVP (0.6 ng/kg/min)	7	113±3.8	364±10.0	6.8±1.0	37.0±4.0
DI i.v. DDAVP (0.2 ng/kg/min)	7	111±9.6	380±9.3	7.3±0.4	36.9±3.8
DI i.c.v. AVP (2 pg/kg/min)	13	115±4.5	364±9.7	8.5±1.1	37.3±3.4
DI i.c.v. DDAVP (2 pg/kg/min)	6	130±4.6	387±8.1	7.5±0.6	39.7±2.5
DI i.c.v. DDAVP (0.2 ng/kg/min)	6	120±3.8	370±7.9	6.9±0.7	38.8±3.0
LE i.c.v. AVP (2 pg/kg/min)	8	106±3.6	336±10.5	6.8±0.5	41.6±3.6
LE i.v. AVP-A (50 ng/kg/min)	7	99±3.2	326±6.1	5.6±0.2	30.1±2.0
LE i.c.v. AVP-A (3.3 ng/kg/min)	8	101±3.0	359±7.8	6.5±0.8	41.7±5.7

MAP, mean arterial pressure; HR, heart rate; SD, standard deviation or lability; DI, Brattleboro rats; i.v., intravenous; AVP, arginine vasopressin; DDAVP, desmopressin; i.c.v., intracerebroventricular; LE, Long-Evans rats; AVP-A, vasopressin antagonist.

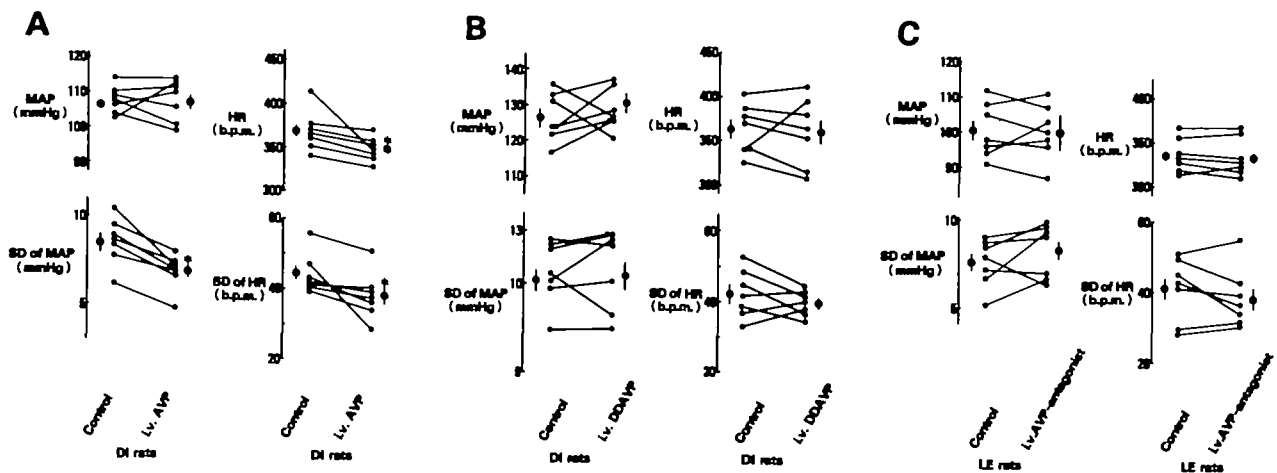


FIGURE 3. Plots showing effects of intravenous arginine vasopressin (AVP) infused at rate of 0.6 ng/kg/min for 21-hour study period (Panel A, n=7) or intravenous desmopressin (DDAVP) infused at rate of 0.2 ng/kg/min for 21 hours (Panel B, n=7) on mean arterial pressure (MAP), heart rate (HR), and standard deviations (SD) of MAP and HR (lability) averaged for 21 hours in Brattleboro (DI) rats. *p<0.05 compared with control. Panel C shows effect of intravenous AVP antagonists infused at rate of 50 ng/kg/min for 21 hours on MAP, HR, and SDs of MAP and HR averaged for 21 hours in Long-Evans (LE) rats (n=7).

Intracerebroventricular desmopressin at a rate of 2 pg/kg/min or 0.2 ng/kg/min did not affect mean arterial pressure, heart rate, or their lability in DI rats. These doses of intracerebroventricular desmopressin significantly decreased the urinary volume from 280±27 to 220±19 ml/21 hr or 318±32 to 48±8 ml/21 hr, respectively.

Intracerebroventricular infusion of the AVP antagonist at a rate of 3.3 pg/kg/min in LE rats produced transient pressor and tachycardiac actions (not significant) with stabilization of heart rate ($F_{1,306}=11.1$, $p<0.001$) during the first few hours. However, it did not affect the lability of blood pressure (Figure 7).

Discussion

The present results demonstrate that mean arterial pressure in DI rats is higher than that in LE rats. This

is in contrast to the results of other workers.¹⁷⁻¹⁹ However, in these earlier studies arterial blood pressure was measured in anesthetized rats, in conscious animals under some degree of stress,¹⁷⁻¹⁹ or was monitored for only a short period.^{9,17} In contrast to the blood pressure difference observed in the present study, there was very little difference in heart rate between the two strains of rats. However, lability of blood pressure and heart rate, which is expressed as the standard deviations of mean arterial pressure and heart rate averaged for the 21-hour study period, was clearly higher in DI than in LE rats.

DI rats have hereditary hypothalamic diabetes insipidus²⁰ with associated polyuria and polydipsia. It may be possible that incessant thirst and drinking may raise blood pressure and induce a high lability of blood pressure and heart rate in DI rats. This is unlikely, however, in view of the finding that desmo-

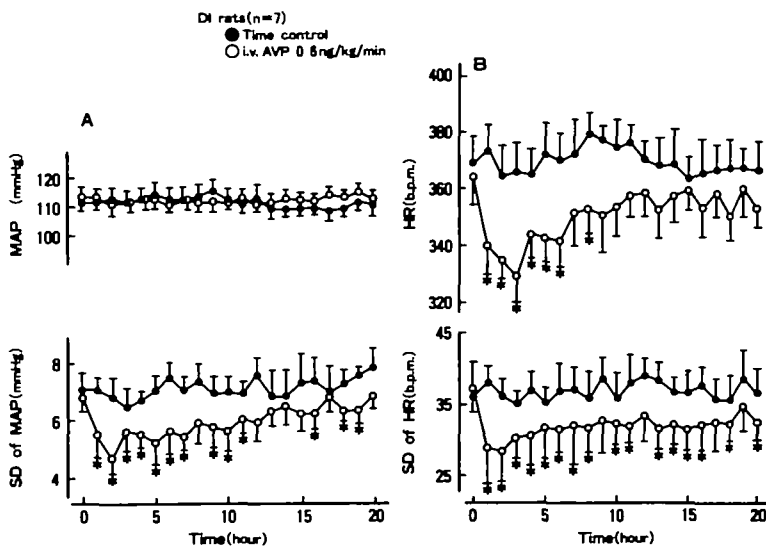


FIGURE 4. Line graphs showing effect of intravenous infusion of arginine vasopressin (AVP) at rate of 0.6 ng/kg/min for 21 hours on mean arterial pressure (MAP) (Panel A), heart rate (HR) (Panel B) and standard deviations (SD) of MAP and HR (lability) averaged every hour in Brattleboro (DI) rats. ●, Represent results of time control experiment obtained during intravenous infusion of physiological saline. ○, Represent effects of intravenous AVP. *p<0.05 against control (0 hour) by Duncan's multiple range test.

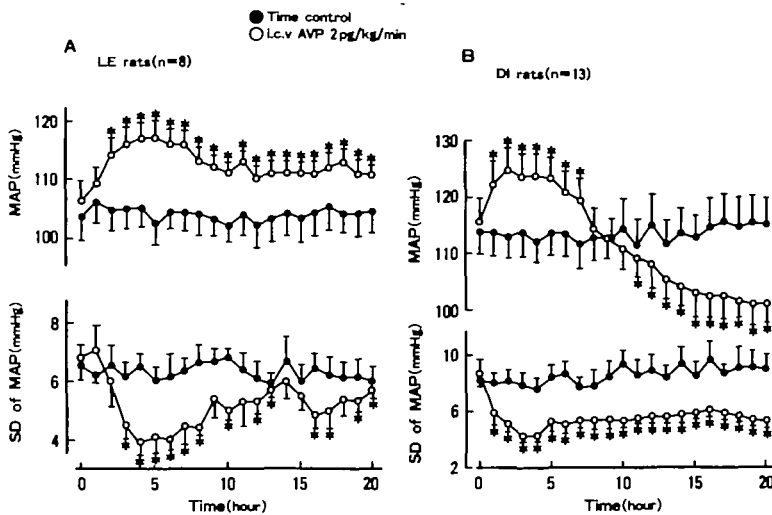


FIGURE 5. Line graphs showing effect of intracerebroventricular infusion of arginine vasopressin (AVP) at rate of 2 pg/kg/min for 21-hour study period on mean arterial pressure (MAP) and its standard deviation (SD) averaged every hour in Long-Evans (LE) rats (Panel A) and Brattleboro (DI) rats (Panel B). ●, Represent results of time control experiment obtained during intracerebroventricular infusion of artificial cerebrospinal fluid. ○, Represent effect of intracerebroventricular AVP. *p<0.05 compared with control (0 hour) by Duncan's multiple range test.

pressin, which completely inhibited polyuria, did not affect the higher blood pressure and the greater lability of blood pressure and heart rate in DI rats.

AVP is one of the most potent vasoconstrictor agents known¹ and also increases body fluid volume by its antidiuretic action on the kidney. In view of these physiological effects, the absence of the actions of AVP on the peripheral circulation of DI rats might be expected to result in hypotension rather than hypertension. It has been shown, however, that the vasoconstrictor action of vasopressin works as a small part of an integrated neurohormonal blood pressure regulation system, which includes the sympathetic nervous system, renin-angiotensin system, and many other regulators.²¹ When one system is removed, its influence can be partially and sometimes completely compensated by increased activity in the remaining systems.^{22,23} Thus, the deficiency of circulating AVP in DI rats may not necessarily result in hypotension, but the relatively higher blood pressure levels observed in DI rats are not easily explained. Several possible mechanisms may be postulated. It is known

that the activity of the renin-angiotensin system is enhanced in DI rats, possibly as a means of compensating for the absence of AVP.²⁴ However, under the same experimental conditions as applied in the present study, Hiwatari et al²³ demonstrated that, after total autonomic blockade, blood pressure in DI rats was lower than that in LE rats, and the renin-angiotensin system was activated more by autonomic blockade in the former. Therefore, higher blood pressure in DI rats may not be attributable to enhanced activity of the renin-angiotensin system.

It has been shown that AVP has a specific and direct effect on modulating baroreceptor reflexes.⁶⁻¹² Earlier studies have demonstrated that DI rats have a depressed baroreceptor reflex function that can be corrected by intravenous infusion of AVP or desmopressin.⁹ The depressed baroreceptor reflex function in DI rats is also supported by the fact that the lability of blood pressure and heart rate in DI rats was greater than that in LE rats, and the exaggerated lability was diminished by intravenous or intracerebroventricular AVP. This effect of intracerebroven-

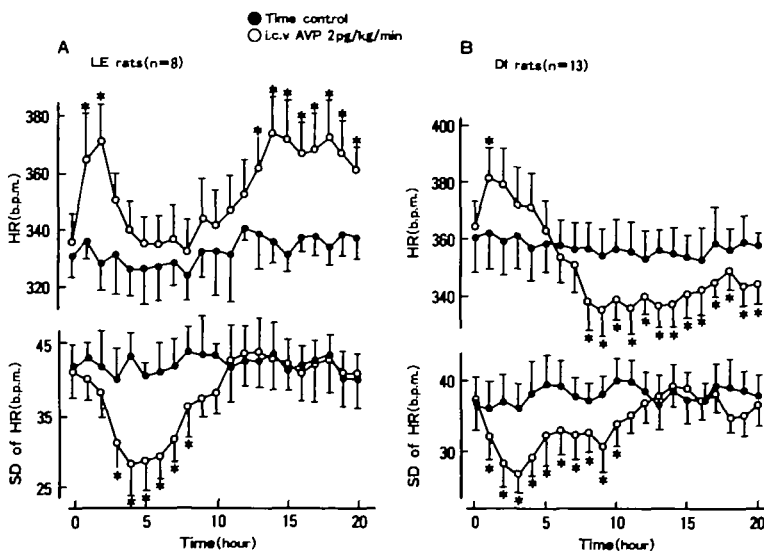


FIGURE 6. Line graphs showing effect of intracerebroventricular infusion of arginine vasopressin (AVP) at a rate of 2 pg/kg/min for 21-hour study period on heart rate (HR) and its standard deviation (SD) averaged every hour in Long-Evans (LE) rats (Panel A) and Brattleboro (DI) rats (Panel B). ●, Represent results of time control experiment obtained during intracerebroventricular infusion of artificial cerebrospinal fluid. ○, Represent effect of intracerebroventricular AVP. *p<0.05 compared with control (0 hour) by Duncan's multiple range test.

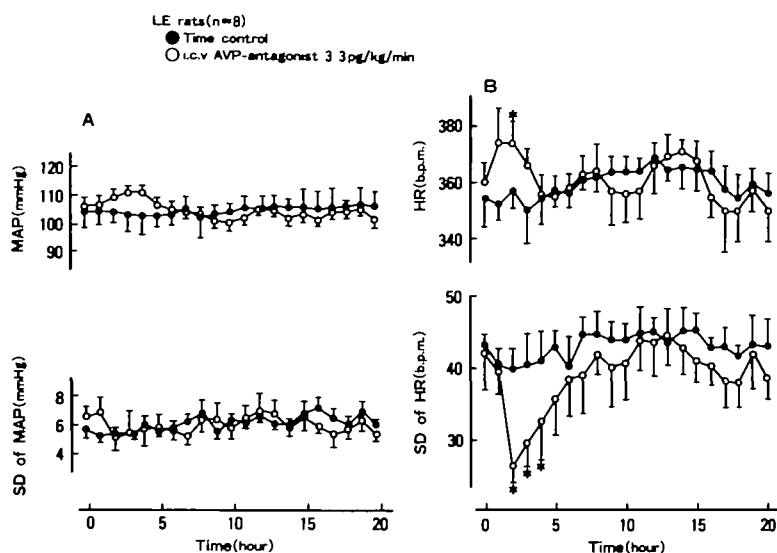


FIGURE 7. Line graphs showing effect of intracerebroventricular infusion of the arginine vasopressin (AVP) antagonist $d(CH_2)_5$ Tyr(Me)-AVP at rate of 3.3 pg/kg/min for the 21-hour study period on mean arterial pressure (MAP), heart rate (HR), and standard deviations (SD) of MAP and HR averaged every hour in Long-Evans (LE) rats. ●, Represent result of time control experiment obtained during intracerebroventricular infusion of artificial cerebrospinal fluid. ○, Represent effect of intracerebroventricular AVP antagonist. * $p < 0.05$ against control (0 hour) by Duncan's multiple range test.

tricular AVP could not be explained by its peripheral action after leakage from the cerebral ventricles; the same amount of intravenous AVP (2 pg/kg/min) did not modulate the lability of blood pressure and heart rate. If we hypothesize that the depressed baroreceptor reflex function in DI rats results in a higher level of blood pressure, the question arises as to why intravenous AVP did not induce hypotension through its activating effect on the baroreceptor reflexes. Our observation was that neither a sufficiently suppressor dose of intravenous AVP (0.1 ng/kg/min) nor a higher dose (0.6 ng/kg/min) caused any change in blood pressure. Thus, it is unlikely that the depressor effect produced by activating baroreceptor reflex is counterbalanced by the pressor effect of exogenous AVP. The present study also does not clarify the reason why intravenous desmopressin, which potentiates baroreceptor reflex sensitivity in DI rats,⁹ did not stabilize blood pressure and heart rate.

Another possibility to explain the relatively higher blood pressure in DI rats is that this is due to the deficiency of central AVP. It has been repeatedly confirmed that there are extensive extrahypothalamic vasopressinergic axonal projections from the hypothalamic AVP-producing nuclei, the supraoptic nucleus (SON) and paraventricular nucleus (PVN), to the brainstem cardiovascular centers.^{25,26} Williams and Johnson²⁷ reported that the activity of the sympathetic nervous system in DI rats is elevated, and they hypothesized that the elevated sympathetic nerve activity in this strain is mediated by the deficiency of AVP in the central nervous system. Thus, it is possible that the high blood pressure in DI rats is mediated by a deficiency of a central vasopressinergic system by which tonic cardiovascular inhibition is maintained. The present results may support the hypothesis that the deficiency of central AVP is responsible for higher blood pressure and higher lability of blood pressure and heart rate in DI rats because intracerebroventricular AVP infusion to DI

rats lacking endogenous AVP induced hypotension and bradycardia. In addition to these effects, intracerebroventricular AVP also dramatically reduced the lability of blood pressure and heart rate in both strains of rats.

In previous studies, centrally administered AVP has been reported to cause hypertension and tachycardia in rats.²⁸⁻³⁰ The pressor and tachycardiac effects of intracerebroventricular AVP reported previously or observed also in the present study might be mediated by the midbrain or brainstem cardiovascular centers because microinjection of AVP to the nucleus tractus solitarius (NTS) or locus coeruleus also produced hypertension and tachycardia.^{31,32} Thus, it is possible to postulate that central AVP acts as a neurotransmitter or neuromodulator in cardiovascular regulatory centers, and may, directly or indirectly, induce both pressor and depressor effects. It has been reported that the central administration of lysine vasopressin to dogs or cats caused decreases in blood pressure.^{33,34} Furthermore, Brattström et al³⁵ recently observed the hypotensive and bradycardic effects of a picogram range of vasopressin administered into the NTS of rats. We also observed that protracted infusion of a small amount of intracerebroventricular AVP induced long-lasting hypotension and bradycardia in stroke-prone SHR, whereas it induced hypertension and tachycardia in WKY rats.¹⁵ Several authors have reported a decreased AVP content in brain nuclei of SHR.³⁶⁻³⁸ It has also been reported that central vasopressinergic neuronal mechanisms contribute to attenuating the centrally mediated pressor effects of stimulation of mesencephalic reticular formation.^{39,40} These findings suggest that central vasopressinergic mechanism may act, at least partly, as a vasodepressor neurotransmitter or neuromodulator. In view of this, it is possible that the hypotensive and bradycardic effects of intracerebroventricular AVP in DI rats reported in the present study as well as in SHR reported previously¹⁵ are mediated by the action of AVP at

loci where AVP acts tonically to cause cardiovascular depression. The same hypothesis may explain the relatively high blood pressure in DI rats, which can be attributed to the deficiency of cardiovascular inhibition by central AVP as expected in SHR.¹⁵ In LE rats, endogenous AVP may occupy the region where exogenous AVP induces cardiovascular depression in DI rats; additional AVP stimulation would cause no further decrease in blood pressure and heart rate. The reason why the heart rate in DI rats was not higher than that in LE rats warrants further study.

A final issue worthy of discussion is the dissociation of the effects of AVP, desmopressin, and AVP antagonist on cardiovascular parameters. In the present study, intravenous or intracerebroventricular AVP, both V₁ vascular and V₂ renal receptor agonist, lowered blood pressure or heart rate and diminished the lability of blood pressure and heart rate in DI rats. However, intravenous or intracerebroventricular desmopressin, which is the preferential V₂ renal receptor agonist,⁴¹ did not. Thus, it seems that hypertension or bradycardia and lability of blood pressure and heart rate are not mediated through a V₂ receptor mechanism. Furthermore, the intravenous or intracerebroventricular V₁ receptor antagonist *d*(CH₂)Tyr(Me)-AVP,¹⁶ modulated neither the lability of blood pressure and heart rate nor basal blood pressure and heart rate in LE rats. The doses of intracerebroventricular and intravenous AVP antagonist used in the present study (3.3 pg/kg/min and 50 ng/kg/min, respectively) may be enough to block the effect of endogenous AVP. Certainly intravenous AVP antagonist was sufficient to abolish the effect of a huge amount of intravenous AVP administered as a single bolus. It can be calculated that the AVP antagonist dose/AVP dose ratio is higher for the intracerebroventricular (1.7) than the intravenous route (0.5) of administration. Because blockade was demonstrated for the intravenous protocol, it could be argued that, in view of the higher dose ratio, this dose should be adequate blockade for the intracerebroventricular protocol. Also, because the pressor effect of AVP was still completely inhibited even after a few hours of infusion of AVP antagonist (the data are not presented), the half-life of AVP antagonist may be extremely longer than that of AVP. Thus, it is hypothesized that intracerebroventricular AVP antagonist at a rate of 3.3 pg/kg/min would attain a CSF concentration level high enough to block the effect of AVP in CSF. Although it is still uncertain whether the AVP antagonist can block the effect of endogenous AVP within the central nervous system, these results may indicate that the receptor mechanism that mediates the effects on blood pressure and heart rate is not identical to the classic V₁ receptor. It has been reported previously that the AVP receptor in the central nervous system might well differ from both V₁ and V₂ receptors.^{42–45} Analogues of AVP that do not possess the classic peripheral effects of AVP still exhibit central activity.⁴³ In

the present study, intracerebroventricular AVP antagonist did not change blood pressure, heart rate, and the lability of blood pressure and heart rate, but rather stabilized the heart rate in LE rats. It is possible that the agonistic action of the AVP antagonist⁴⁶ or its metabolite may act in the central nervous system to produce AVP-like effects. These results, however, warrant a more systematic exploration of the mechanism for the centrally mediated cardiovascular effect of AVP.

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KEY WORDS • arginine vasopressin • blood pressure • central nervous system • heart rate • Brattleboro rat • Long-Evans rat