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# Restoration of Suppressed Baroreflex Sensitivity in Rats with Hereditary Diabetes Insipidus (Brattleboro Rats) by Arginine-Vasopressin and DDAVP

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**SUMMARY.** To determine the influence of vasopressin on baroreceptor reflex mechanisms, baroreflex function in Brattleboro rats was compared with that in normal Long-Evans rats. Baroreflex function was assessed in conscious unrestrained rats during increases in blood pressure with phenylephrine (200  $\mu\text{g}/\text{kg}$  per min for 10 seconds). The baroreflex function line was obtained by plotting the log (pulse period) against the preceding systolic pressure on a beat-by-beat basis. The slope of the baroreflex function line (baroreflex function slope) in Long-Evans rats [ $(19.0 \pm 1.4) \times 10^{-4}$ , mean  $\pm$  SEM,  $n = 34$ ] was significantly steeper than that in Brattleboro rats [ $(6.9 \pm 0.6) \times 10^{-4}$ ,  $n = 44$ ,  $P < 0.001$ ]. A subpressor intravenous infusion of arginine<sup>8</sup>-vasopressin (2 ng/kg per min for 2 hours), which elevated plasma vasopressin to  $48.1 \pm 6.8$  pg/ml, caused bradycardia and increased the baroreflex function slope in Brattleboro rats, from  $(7.5 \pm 1.0) \times 10^{-4}$  to within the normal Long-Evans range [ $(17.0 \pm 0.8) \times 10^{-4}$ ,  $n = 7$ ,  $P < 0.001$ ]. The basal pulse period and the baroreflex function slope in Brattleboro rats [ $(7.0 \pm 0.9) \times 10^{-4}$ ] was also increased significantly to  $(12.0 \pm 1.7) \times 10^{-4}$  ( $n = 11$ ,  $P < 0.01$ ) by an infusion of 1-desamino-8-D-arginine vasopressin, (2 ng/kg per min for 2 hours), a vasopressin analogue with potent antidiuretic but minimal vascular actions. Acute volume expansion, which increased body weight significantly, did not change the baroreflex function slope in Brattleboro rats [ $(7.7 \pm 1.1) \times 10^{-4}$  vs.  $(8.2 \pm 1.7) \times 10^{-4}$ ,  $n = 6$ ]. A specific vasopressin vascular receptor antagonist [d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)AVP], although blocking the pressor effect of exogenous vasopressin, did not change the pulse period or the baroreflex function slope [ $(16.0 \pm 2.3) \times 10^{-4}$  vs.  $(19.0 \pm 1.7) \times 10^{-4}$ ,  $n = 6$ ] in normal Long-Evans rats. The results obtained in Brattleboro rats and the change in baroreflex sensitivity brought about by infusions of vasopressin and DDAVP provide strong evidence that vasopressin may be an important physiological modulator of baroreflex function. (*Circ Res* 53: 140-149, 1983)

SEVERAL recent findings indicate that the vasoconstrictor property of vasopressin may have physiological significance. Although low infusion rates of vasopressin which produce plasma levels that are close to the physiological range can elevate blood pressure (Szczepanska-Sadowska, 1973; Pullan et al., 1980), circulatory reflex mechanisms normally buffer the arterial pressure increase following vasopressin vasoconstriction (Johnston et al., 1981). The pressor response to vasopressin is remarkably potentiated when reflex buffering mechanisms are eliminated by sinoaortic denervation (Cowley et al., 1974) or pharmacological blockade of the sympathetic nervous system (Pullan et al., 1980). Furthermore, Cowley et al. (1974) and Matsuguchi and Schmid (1982) have demonstrated that after elimination of baroreflex buffering, the pressor response to vasopressin was augmented more than those to phenylephrine or norepinephrine. It was also reported recently that even small elevations in plasma vasopressin caused considerable bradycardia without change in arterial pressure (Pullan et al., 1980; Möhring et al., 1981). Möhring et al. (1981) also demonstrated that heart rate fell more for a given

increase in arterial pressure during infusion of vasopressin than during an infusion of phenylephrine or norepinephrine. These results imply that vasopressin specifically modulates the baroreflex pathway. Whether these are peripherally or centrally mediated effects of vasopressin is not known. Recent studies suggest that vasopressin and/or central vasopressinergic neurones can alter the function of the baroreflex or that of central cardiovascular control systems. Centrally administered vasopressin causes hypertension and tachycardia in rats (Matsuguchi et al., 1982; Pittman et al., 1982; Imai et al., 1983). Several recent reports also showed that centrally administered vasopressin increased the baroreflex sensitivity in rats (Izdebska et al., 1982; Imai et al., 1983). On the other hand, Brattström and Kalkoff (1970) reported, to the contrary, that intracisternal administration of vasopressin attenuated the blood pressure lowering effect of carotid sinus stimulation in dogs, suggesting suppressed inhibition of central sympathetic vasomotor activity. Extra-hypothalamic-pituitary vasopressinergic neurones project to some regions of the central vasomotor centers (Saper et al., 1976; Buijs et al., 1978; Sofroniew, 1980).

Berecek et al. (1982) reported that stimulation of the paraventricular nucleus, a vasopressin synthesizing nucleus, produced frequency dependent bradycardia and hypotension in rats. However, it was also reported that stimulation of vasopressin synthesizing nuclei attenuates the bradycardia produced by carotid sinus stimulation in cats (Ciriello and Calaresu, 1980). Intra-cisternal administration of vasopressin also attenuated the blood pressure-lowering effect of carotid sinus stimulation in dogs (Brattström and Kalkoff, 1970). Thus, under normal conditions, endogenous vasopressin may have circulatory effects both by direct vasoconstriction and by a baroreflex mechanism. In the present study, the role of endogenous vasopressin in influencing the baroreceptor reflex mechanism has been examined by comparing baroreflex function in Brattleboro rats with hereditary hypothalamic diabetes insipidus with that in Long-Evans rats of the parent strain. The mechanism of action of vasopressin on baroreflex function also was studied.

### Methods

Male homozygous Brattleboro rats with hereditary hypothalamic diabetes insipidus (DI rats) and male Long-Evans rats of the parent strain (LE rats) weighing 200–400 g and aged 24–27 weeks were used. All rats were allowed free access to chow and water until the surgical procedure. Under ether anaesthesia, the left femoral artery and vein were catheterized, using polyethylene tubing. The tip of the arterial catheter (Intramedic PE100; Clay Adams) was tapered by heating for insertion into the femoral artery. Intramedic PE50 was used as a venous catheter. In some rats, the right femoral vein was also catheterized for the continuous infusion of drugs or 5% dextrose solution. The catheters were passed subcutaneously and brought out on the neck. Catheters were filled with heparinized saline (1000 IU/ml) and sealed by heating. During surgery, 15 mg of aminobenzyl penicillin were administered topically and intraperitoneally. Rats were allowed to recover for at least 24 hours after surgery and were conscious and unrestrained during subsequent studies.

During each experiment, rats were placed in rectangular boxes (30 × 17 × 17 cm) without any restriction of movement, and were allowed free access to water. Blood pressure was recorded from the femoral arterial catheter using a P23Db Statham pressure transducer. Pulse period was monitored with a period meter (Baker Institute model Unicon kfh 122B). Both parameters were recorded continuously on a Brush recorder (model 440). One hour was allowed for stabilization of the blood pressure and pulse period before measurement of baroreflex function. The arterial pressure dose-response curves for several vasopressor substances were obtained.

The drugs used in the present study were: phenylephrine hydrochloride (Sigma), (Arg<sup>8</sup>)-vasopressin (Calbiochem), 1-desamino-8-D-arginine vasopressin (DDAVP, Minirin, Ferring AB), Pmp<sup>1</sup>, O-methyl-Tyr<sup>2</sup>-(Arg<sup>8</sup>)-vasopressin [d(CH<sub>2</sub>)<sub>5</sub>Tyr(Me)AVP, Peninsula Laboratories], angiotensin II (Hypertensin, Ciba) and atropine sulfate (David Bull Laboratories). Stock solutions of arginine-vasopressin (AVP) and its analogues were prepared in 0.1 N acetic acid. Phenylephrine and angiotensin II were dissolved in 0.9% saline. All drug solutions were diluted

to the desired concentrations with 0.9% saline. The solution of phenylephrine was infused at rates up to 22  $\mu$ l/sec by an infusion pump (Harvard apparatus model 660-910/920). Solutions of vasopressin or its analogues were infused at a rate of 30  $\mu$ l/min by an infusion pump (DELTA model MHRE 22). Infusion of an equivalent volume of vehicle was shown to have no significant cardiohemodynamic effects. All drug solutions were injected or infused through the implanted venous catheter.

### Measurement of Baroreflex Function

Baroreflex function was assessed in conscious unrestrained rats by pharmacological increases of blood pressure with phenylephrine, according to the method of Smyth et al. (1969). Phenylephrine was infused at a rate of 200  $\mu$ g/kg per min for 10 seconds to obtain nearly maximum pressor effect without marked irregularity in pulse period. Pulse period (log scale) was plotted against systolic blood pressure on a beat-by-beat basis to provide the baroreflex function curve. Only those points that were located on the linear portion of this curve were used to calculate what we have termed the "baroreflex function line" (Fig. 1). The sensitivity of the reflex was determined by the slope of baroreflex function line (baroreflex function slope). The slope was calculated from the plot of the systolic blood pressure and log (pulse period) using regression analysis and expressed as follows:

Slope =  $\Delta \log PP / \Delta SBP$ , where PP is pulse period (msec) and SBP is systolic blood pressure (mm Hg).

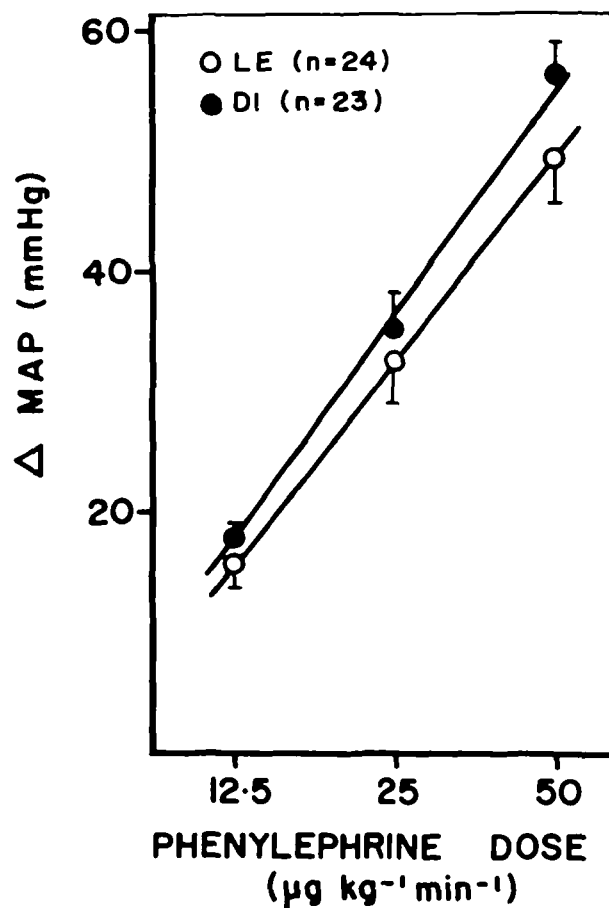


FIGURE 1. Pressor dose-response curves against phenylephrine in LE and DI rats.

The sigmoid curve which depicts a complete baroreflex function was not obtained in the present experiments. Decreasing blood pressure with nitroprusside caused only a very small decrease in pulse period in the conscious rat (unpublished data); these points did not fall on the linear portion of the baroreflex function line and were therefore not included in the analysis.

### Experimental Protocols

Experiments were performed as described below. Each rat was studied twice with an interval of at least 48 hours between successive experiments. The age, body weight, control values of mean arterial pressure, pulse period, and heart rate are shown in Table 1.

#### Experiment 1. Difference of Baroreflex Sensitivity between LE and DI Rats

Baroreflex function was determined in 34 LE and 44 DI rats. As a preliminary experiment, the pressor-dose response curve to phenylephrine at rates of 12.5, 25, and 50  $\mu\text{g}/\text{kg}$  per min for 1 minute was examined in 24 LE rats and 23 DI rats. At least 10 minutes were allowed between increasing doses of phenylephrine. The time to attain the peak pressor response was calculated.

#### Experiment 2. Effect of Subpressor Dose of AVP on Baroreflex Function in DI Rats

To determine the subpressor dose of AVP, the pressor-dose response relationship of AVP at rates of 30, 60, and 120  $\text{ng}/\text{kg}$  per min for 1 minute was examined in 27 LE rats and 15 DI rats. The subpressor dose of AVP was determined by extrapolation of this dose-response curve.

The baroreflex function was examined in seven DI rats before and during infusion of the subpressor dose of AVP. After establishing a control baroreflex function line, AVP was infused at a rate of 2  $\text{ng}/\text{kg}$  per min for 2 hours, and baroreflex function was reexamined during AVP infusion. At the conclusion of this experiment, the rat was decapitated and blood was collected for measurement of plasma vasopressin.

#### Experiment 3. Effect of DDAVP on Baroreflex Function in DI Rats

The baroreflex function was examined in 11 DI rats before and during infusion of DDAVP, a vasopressin analogue with potent antidiuretic activities and with minimal cardiovascular effects. After control baroreflex function had been determined, DDAVP was infused at a rate of 2  $\text{ng}/\text{kg}$  per min for 2 hours, and baroreflex function was reexamined during this period.

#### Experiment 4. Effect of Volume Expansion on Baroreflex Function in DI Rats

The effect of acute volume expansion on baroreflex function was examined in six DI rats. After control baro-

reflex function had been measured, an initial injection of 15 ml/kg of 5% dextrose solution was made, followed by infusion of a 5% dextrose solution at 1 ml/kg per min for 2 hours. The baroreflex function was reexamined during volume expansion. Body weight and arterial blood hematocrit were measured before infusion and just after the second measurement of baroreflex function.

#### Experiment 5. Effect of a Specific Vasopressin Vascular Receptor Antagonist on Baroreflex Function in LE Rats

The effect of the vasopressin specific vascular receptor antagonist,  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  (Kruszynski et al., 1980), on cardiohemodynamics was examined in nine LE rats. The baroreflex function was examined in six of these animals before and during infusion of  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ . After testing the control baroreflex function,  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  was injected in a dose of 1  $\mu\text{g}/\text{kg}$  followed by infusion at 5  $\mu\text{g}/\text{kg}$  per hr for 2 hours. Baroreflex function was reexamined during this period. To establish that this dose of antagonist was effective in these rats, the pressor effect of vasopressin in a dose of 100  $\text{ng}/\text{kg}$  was examined before and during  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  treatment.

#### Experiment 6. Effect of Combined Treatment with AVP and Specific Vasopressin Vascular Receptor Antagonist on Baroreflex Function in DI Rats

The baroreflex function was examined in five DI rats before and during combined treatment with AVP and specific vasopressin vascular receptor antagonist. After testing the control baroreflex function, AVP was infused at a rate of 2  $\text{ng}/\text{kg}$  per min for 2 hours, and baroreflex function was reexamined during AVP infusion. Thereafter, vasopressin vascular receptor antagonist,  $\text{d}(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ , was administered in addition to AVP. The dose and procedure of administration of antagonist is the same as that stated previously. The baroreflex function was reexamined during combined treatment with AVP and antagonist.

#### Experiment 7. Effect of Atropine on Baroreflex Sensitivity in LE and DI Rats

To determine the parasympathetic contribution to the baroreflex in rats, the effect of atropine was examined in six LE and six DI rats. After testing the control baroreflex function, we injected 1  $\text{mg}/\text{kg}$  of atropine sulphate, intravenously, and 15 minutes later, again examined baroreflex function.

### Analytical Methods

Plasma vasopressin concentration was measured in six DI rats with and without infusion of AVP at a rate of 2  $\text{ng}/\text{kg}$  per min for 2 hours. Plasma vasopressin concentration was determined by radioimmunoassay (Woods and Johnston, 1982).

TABLE 1  
Age, Body Weight, and Basal Values of Mean Arterial Pressure, Pulse Period, and Heart Rate in LE and DI Rats

Rat	n	Age (wks)	Body wt (g)	MAP (mm Hg)	Pulse period (msec)	Heart Rate (beats/min)
LE	34	23.8 $\pm$ 1.2	327 $\pm$ 8	96 $\pm$ 2	150 $\pm$ 3	407 $\pm$ 9
DI	44	26.5 $\pm$ 1.2	313 $\pm$ 6	100 $\pm$ 4	164 $\pm$ 4†	375 $\pm$ 4*

\* Significant difference from LE rat ( $P < 0.01$ ); †  $P < 0.001$ .

Antibodies to synthetic arginine-vasopressin were raised in New Zealand White rabbits after conjugating the peptide to bovine serum albumin by glutaraldehyde. The dilution of antiserum that bound 50% of the tracer was a final dilution of 1:120,000. The antibody cross-reacted 10% with synthetic lysine-vasopressin, 100% with DDAVP, and less than 0.001% with synthetic oxytocin, arginine vasotocin, and human neurophysins I and II. Synthetic AVP (Ferring, A.B., Malmo, Sweden) was used as standard. The rats were decapitated and blood was collected into chilled heparinized tubes and, after centrifugation, the plasma was separated, stored at  $-20^{\circ}\text{C}$ , and extracted with acetone/petroleum ether. The mean recovery of AVP was  $83.2 \pm 2.8\%$ , and was linear over the range of 0–20 pg. The assay sensitivity was 0.6 pg/ml and the intra- and interassay variability were 8.4% and 11.6%, respectively.

#### Statistical Methods

All values reported are the mean  $\pm$  SEM unless otherwise stated. The dose-response curves for phenylephrine in LE and DI rats were compared, using analysis of variance. Linear regression equations were calculated by the method of least squares. The slopes of the regression line for baroreflex function within a group were compared by Student's *t*-test for paired comparison, and those between groups were analyzed by one-way analysis of variance.

### Results

#### Age, Body Weight, and Basal Values of Blood Pressure and Pulse Period in LE and DI rats

As shown in Table 1, the body weight of DI rats was slightly lower than that of LE rats, although the DI rats were older. However, these differences were not statistically significant. Although there was no significant difference in basal mean arterial pressure between LE and DI rats, the basal pulse period in DI rats was significantly longer ( $P < 0.001$ ) than that in LE rats. Hence, conscious unrestrained DI rats had a slower heart rate than LE rats.

#### Experiment 1. Baroreflex Sensitivity in LE and DI Rats

There was no difference between the pressor dose-response curves to phenylephrine in LE and DI rats (Fig. 1). The time to attain peak mean arterial pressure in response to  $50 \mu\text{g}/\text{kg}$  per min of phenylephrine in DI rats ( $26.2 \pm 2.3$  seconds) was also not significantly different from that in LE rats ( $26.1 \pm 1.7$  seconds).

Figure 2 illustrates a typical plot obtained after phenylephrine infusion in a LE and DI rat over a blood pressure range of 100–200 mm Hg. Figure 3 (left) compares the basal mean arterial pressure and pulse period and average calculated baroreflex function line for LE and DI rats. All baroreflex function slopes from LE and DI rats were plotted and together with the mean  $\pm$  1 sd of the slopes, are illustrated in Figure 3 (right). The baroreflex function slope in LE rats [ $(19.0 \pm 1.4) \times 10^{-4}$ ] was significantly steeper than that in DI rats [ $(6.9 \pm 0.6) \times 10^{-4}$ ,  $F_{1,76} = 73.5$ ,  $P < 0.001$ ]. The baroreflex sensitivity in LE rats was higher than that in DI rats, and hence the baroreflex

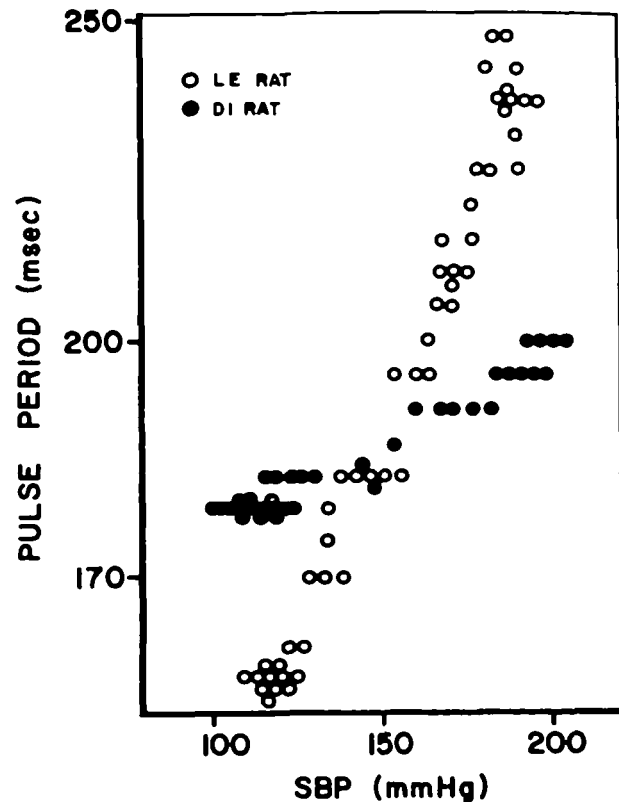


FIGURE 2. Relationship between systolic blood pressure (SBP) and pulse period obtained following phenylephrine infusion in a LE (open circle) and DI (closed circle) rat. Vertical axis is plotted as log scale.

sensitivity is suppressed in Brattleboro rats which lack vasopressin. Thus, for any given rise in blood pressure, less bradycardia was seen in DI than LE rats. The correlation coefficient between the changes in pulse period and systolic blood pressure in LE rats ( $0.91 \pm 0.01$ ) was significantly greater than that in DI rats ( $0.70 \pm 0.04$ ,  $P < 0.001$ ), implying greater variability in pulse period in the absence of AVP.

#### Experiment 2. Effect of a Subpressor Dose of Vasopressin on Baroreflex Function in DI Rats

Arginine-vasopressin at a rate of 2 ng/kg per min for 2 hours did not significantly change the mean arterial pressure ( $98 \pm 3$  vs.  $102 \pm 2$  mm Hg). However, it did significantly prolong the pulse period ( $165 \pm 9$  vs.  $183 \pm 11$  msec,  $P < 0.01$ ). DI rats had no measurable vasopressin in their plasma. The AVP infusion, 2 ng/kg per min, produced a plasma AVP level of  $48.1 \pm 6.8$  pg/ml after 2 hours in DI rats. Figure 4 (left) shows the average calculated baroreflex function lines before and during vasopressin infusion. The regression line was shifted upward during AVP infusion. The individual baroreflex function slopes obtained from each experiment are shown in Figure 4 (right). The baroreflex function slope during AVP infusion [ $(17.0 \pm 0.8) \times 10^{-4}$ ] was significantly steeper than that before AVP infusion [ $(7.5 \pm 1.0) \times 10^{-4}$ ,  $P < 0.001$ ] and not different to that obtained in normal LE rats [ $(19.0 \pm 1.4) \times 10^{-4}$ ,  $F_{1,39} = 0.24$ ,  $P > 0.05$ ]. During AVP

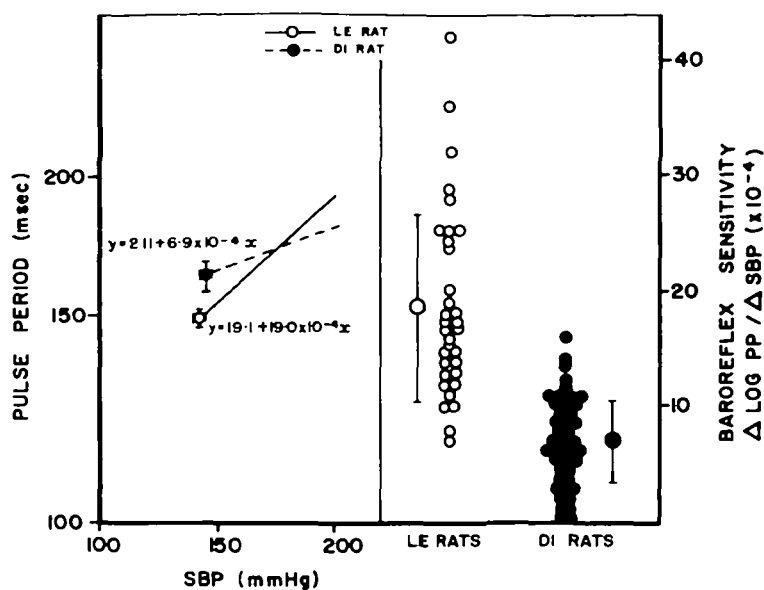


FIGURE 3. Baroreflex sensitivity in LE and DI rats. The average calculated baroreflex function lines from LE rats (open circle and solid line) and from DI rats (closed circle and dotted line) are shown in left figure. The symbols in left figure (open and closed circles) are the mean  $\pm$  SEM of pulse period (PP) and systolic blood pressure (SBP), respectively, at basal condition of the experiment. The vertical axis is plotted as log scale. All points and the mean  $\pm$  1 SD of all the baroreflex function slopes (baroreflex sensitivity) from LE rats (open circle) and DI rats (closed circle) are illustrated in right figure.  $\Delta$  SBP: change in systolic blood pressure.

infusion all baroreflex function slopes fell within the range of mean  $\pm$  1SD of the slopes found in LE rats (Fig. 4, right).

### Experiment 3. Effect of DDAVP on Baroreflex Function in DI Rats

Although DDAVP at a rate of 2 ng/kg per min for 2 hours did not change the mean arterial pressure in DI rats ( $102 \pm 3$  vs.  $101 \pm 3$  mm Hg), this dose

of DDAVP significantly increased the pulse period from  $157 \pm 7$  to  $176 \pm 6$  msec ( $P < 0.01$ ). This short infusion of DDAVP did not change body weight ( $300 \pm 11$  g before and  $301 \pm 10$  g after).

Figure 5 (left) shows the average calculated baroreflex function lines before and during DDAVP infusion. The regression line was shifted upward during DDAVP infusion. The individual baroreflex function slopes obtained from each experiment are

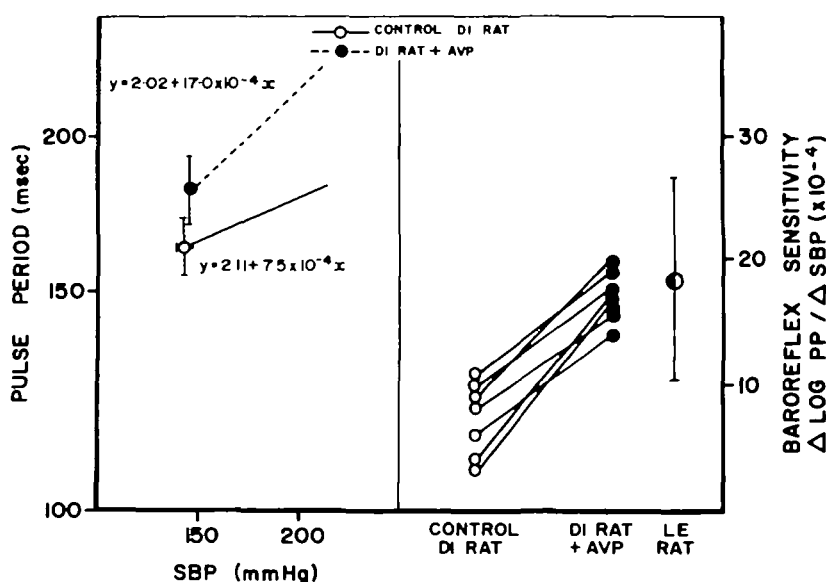


FIGURE 4. Effect of subpressor dose of arginine-vasopressin (AVP; 2 ng/kg per min for 2 hours) on baroreflex function in DI rats. The average calculated baroreflex function lines before (open circle and solid line) and during (closed circle and dotted line) AVP infusions are shown in left figure. All the baroreflex function slopes (baroreflex sensitivity) before (open circle) and during (closed circle) AVP infusion are shown in right figure. Symbol and vertical line in right figure shows the mean  $\pm$  1 SD of the slopes of baroreflex function lines obtained from LE rats. Otherwise, the same as Figure 3.

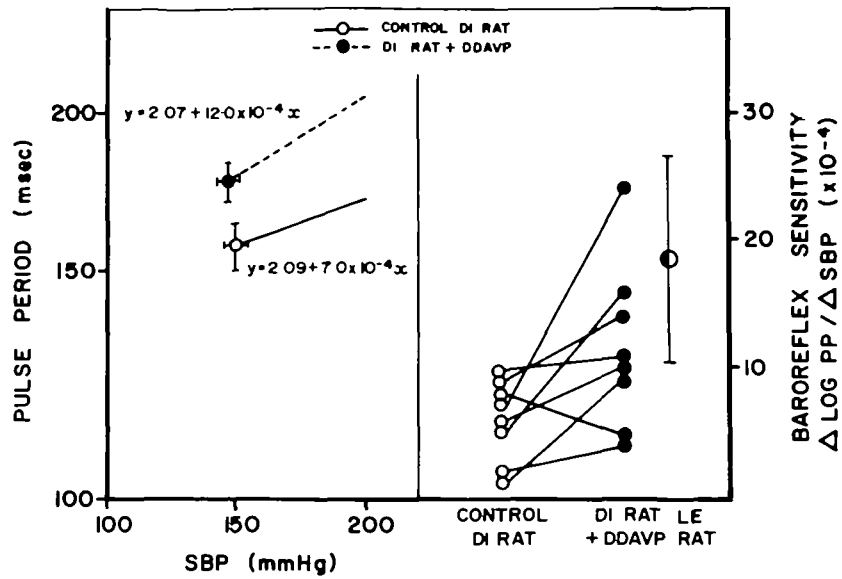


FIGURE 5. Effect of DDAVP on baroreflex function in DI rats. The average calculated baroreflex function line before (open circle and solid line) and during (closed circle and dotted line) DDAVP infusion are shown in left figure. All the baroreflex function slopes (baroreflex sensitivity) before (open circle) and during (closed circle) DDAVP infusion are shown in right figure. Mean  $\pm$  1 sd of slope before and during DDAVP infusion are  $(7.0 \pm 0.9) \times 10^{-4}$  and  $(12.0 \pm 1.7) \times 10^{-4}$ , respectively ( $P < 0.01$ ). Otherwise, the same as Figure 3.

shown in Figure 5 (right). DDAVP increased the slope in seven of eight DI rats. In the remaining one, the baroreflex function slope decreased during DDAVP infusion. As a whole, the baroreflex function slope during DDAVP infusion [ $(12.0 \pm 1.7) \times 10^{-4}$ ] was significantly steeper than that before treatment [ $(7.0 \pm 0.9) \times 10^{-4}$ ,  $P < 0.01$ ]. However, the baroreflex function slope during DDAVP infusion was not as steep as that during AVP infusion [ $(17.0 \pm 0.8) \times 10^{-4}$ ,  $F_{1,16} = 5.62$ ,  $P < 0.05$ ] or that found in LE rats [ $(19.0 \pm 1.4) \times 10^{-4}$ ,  $F_{1,43} = 6.72$ ,  $P < 0.05$ ].

**Experiment 4. Effect of Acute Volume Expansion on Baroreflex Function in DI Rats**

After 2 hours of volume expansion, body weight had increased  $12.2 \pm 5.0$  g ( $P < 0.05$ ) and hematocrit had decreased by  $1.2 \pm 0.2\%$  ( $P < 0.01$ ). However, volume expansion did not change the mean arterial pressure or pulse period.

Figure 6 (left) shows the average calculated baroreflex function lines before and during volume expansion. The individual baroreflex function slopes obtained from each experiment are shown in Figure

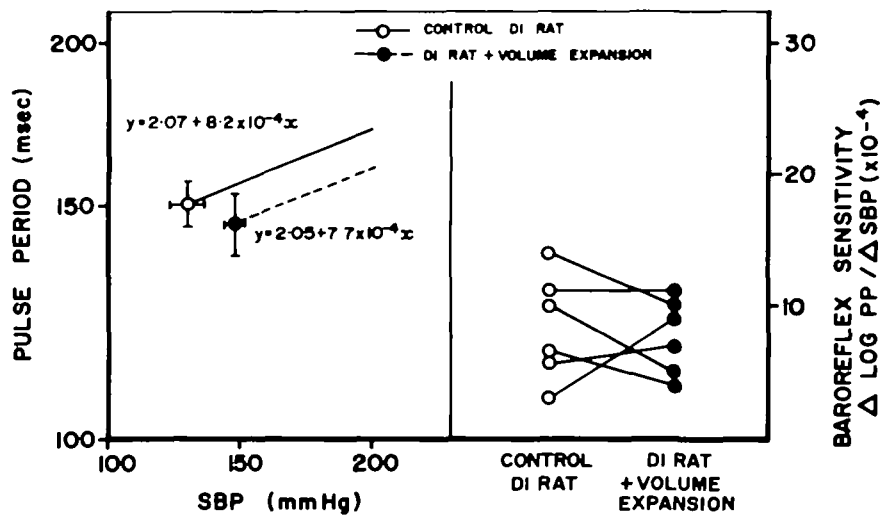


FIGURE 6. Effect of acute volume expansion on baroreflex function in DI rats. The average calculated baroreflex function lines before (open circle and solid line) and during (closed circle and dotted line) volume expansion by 5% dextrose solution are shown in left figure. All the baroreflex function slopes (baroreflex sensitivity) before (open circle) and during (closed circle) volume expansion are shown in right figure. Otherwise, the same as Figure 3.



6 (right). The baroreflex function slope during volume expansion  $[(7.7 \pm 1.1) \times 10^{-4}]$  was not significantly different from that before volume expansion  $[(8.2 \pm 1.7) \times 10^{-4}]$ .

#### Experiment 5. Effect of Vasopressin-Specific Vascular Receptor Antagonist on Baroreflex Function in LE Rats

The vasopressin-specific vascular receptor antagonist,  $d(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$ , increased mean arterial pressure from  $93 \pm 2$  to  $101 \pm 3$  mm Hg in the first 10 minutes ( $P < 0.01$ ) of infusion, but by 2 hours the blood pressure and pulse period were not significantly different from the control values. The pressor effect of AVP at a dose of 100 ng/kg was almost completely inhibited by  $d(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$  ( $49.7 \pm 3.4$  to  $4.0 \pm 1.2$  mm Hg,  $P < 0.001$ ). As shown in Figure 7 (right and left), the baroreflex function slope before treatment  $[(16.0 \pm 2.3) \times 10^{-4}]$  was not significantly different from that during infusion of  $d(\text{CH}_2)_5\text{Tyr}(\text{Me})\text{AVP}$   $[(19.0 \pm 1.7) \times 10^{-4}]$ .

#### Experiment 6. Effect of Vasopressin-Specific Vascular Receptor Antagonist on Baroreflex Function in DI Rats Treated with AVP

The baroreflex sensitivity during AVP infusion  $[(17.0 \pm 0.9) \times 10^{-4}]$  was significantly higher than that in control  $[(9.2 \pm 0.9) \times 10^{-4}]$ . The baroreflex sensitivity during combined treatment with AVP and vasopressin-specific vascular receptor antagonist  $[(17.0 \pm 1.1) \times 10^{-4}]$  was almost identical with that during AVP infusion alone  $[(17.0 \pm 0.9) \times 10^{-4}]$ .

#### Experiment 7. Effect of Atropine on Baroreflex Function in LE and DI Rats

Atropine significantly shortened the resting pulse period both in LE ( $163 \pm 8$  to  $125 \pm 4$  msec,  $P < 0.01$ ) and DI ( $166 \pm 6$  to  $129 \pm 3$  msec,  $P < 0.001$ ) rats, but did not affect mean arterial pressure. In addition, atropine nearly abolished the baroreflex function slope in both LE  $[(18.0 \pm 2.1) \times 10^{-4}]$  to  $(1.0 \pm 0.3) \times 10^{-4}$ ,  $P < 0.001$  and DI rats  $[(6.3 \pm 1.3) \times 10^{-4}]$  to  $(0.8 \pm 0.3) \times 10^{-4}$ ,  $P < 0.001$ .

### Discussion

The present study clearly demonstrates that, in Brattleboro rats with hereditary hypothalamic diabetes insipidus and completely lacking endogenous vasopressin, baroreflex sensitivity is greatly suppressed compared with that in normal Long-Evans rats. Intravenous administration of AVP at a subpressor rate that achieved plasma AVP levels within the physiological range, resulted in bradycardia and restored baroreflex sensitivity toward normal in DI rats. This suggests that vasopressin is a physiological modulator of baroreflex function. The present results demonstrate that endogenous vasopressin makes an important contribution to the maintenance of baroreflex sensitivity in normal rats.

It is widely recognized that systemic administration of vasopressin causes considerable bradycardia in various species of animal. In the present study, intravenous administration of very low amounts of vasopressin into DI rats caused significant bradycardia without change in mean arterial pressure. Since a direct chronotropic effect of vasopressin is observed only when pharmacological doses of vasopressin are administered (Nakashima et al., 1982), the chronotropic effect of vasopressin in low concentration is most likely due to an indirect action mediated by the autonomic nervous system and/or baroreceptor reflexes (Nakano, 1974). Möhring et al. (1981) recently reported that heart rate fell more for a given increase in arterial pressure during infusions of vasopressin than during infusions of phenylephrine or norepinephrine. Several authors also reported that pressor responses to vasopressin were augmented more than those to other vasopressor substances after baroreceptor denervation or autonomic blockade (Cowley et al., 1974; Matsuguchi and Schmid, 1982). These results strongly suggest that vasopressin, unlike most other vasopressor substances, augments baroreflex buffering action.

The mechanism by which vasopressin modulates baroreflex function is not known, but may involve a central action of the peptide. Liard et al. (1981) reported that vasopressin administered selectively into the vertebral artery of dogs induced a lesser increase in mean arterial pressure and a greater decrease in heart rate than the same infusion given intravenously, despite similar increases in plasma vasopressin concentration. They postulated that circulating vasopressin has an effect on a structure in the central nervous system involved in cardiovascular control, possibly by affecting the baroreceptor reflex. However, the centrally mediated cardiovascular actions of vasopressin appear to be complex. The diversity of the results observed may, in part, be due to species difference and the route of administration. Varma et al. (1969) administered vasopressin into the ventricular system of the brain of anesthetized dogs and observed a pronounced slowing of heart rate. This response was inhibited by vagotomy, indicating the involvement of parasympathetic neural pathways. In contrast, Matsuguchi et al. (1982) reported that microinjection of vasopressin into the nucleus tractus solitarius caused tachycardia in anesthetized rats. We have observed that intracerebroventricular infusion of vasopressin at a rate of 2 ng/kg per min causes transient tachycardia in conscious DI rats (Imai et al., 1983), whereas the same dose of vasopressin infused intravenously caused bradycardia. Such results indicate that cardiovascular effects of centrally administered vasopressin are different from those observed after intravenous injection, at least in the rat. It is not yet clear whether circulating vasopressin can cross the blood brain barrier, and since vasopressin may act at several loci within baroreflex pathways, it is difficult to postulate which part (central or peripheral) of the baroreflex pathways may be affected by intravenous

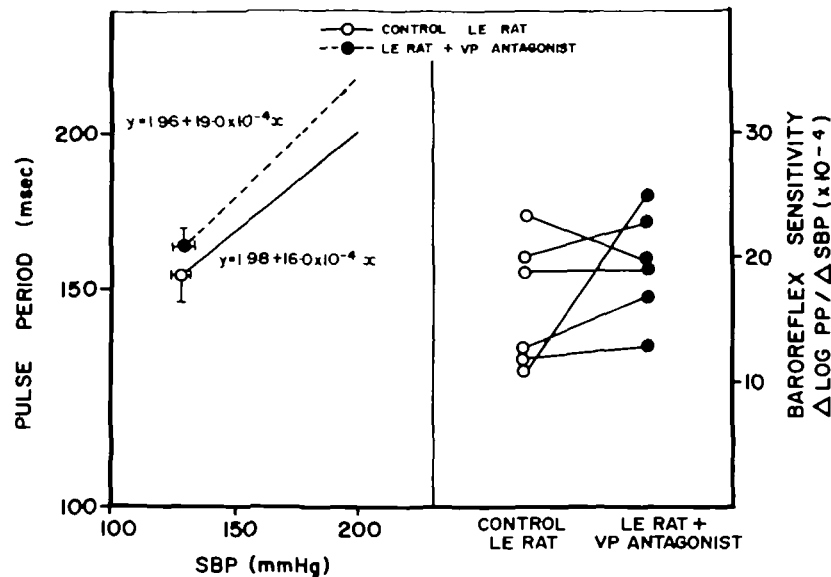


FIGURE 7. Effect of vasopressin-specific vascular receptor antagonist (VP-antagonist) on baroreflex function in LE rats. The average calculated baroreflex function lines before (open circles and solid line) and during (closed circle and dotted line) VP-antagonist are shown in left figure. All the baroreflex function slopes (baroreflex sensitivity) before (open circle) and during (closed circle) VP-antagonist are shown in right figure. Otherwise, the same as Figure 3.

infusions of vasopressin in DI rats. Some effects of circulating vasoactive substances like angiotensin II and prostaglandins are mediated via the central nervous system (Scroop and Lowe, 1969; Ferrario et al., 1970; Lavery et al., 1970; Sweet et al., 1971). Izdebska et al. (1981) reported that intracerebroventricular injection of lysine-vasopressin into anesthetized rats enhanced reflex bradycardia induced by a blood pressure rise. We also observed enhanced baroreflex sensitivity by central infusion of vasopressin in conscious DI rats (Imai et al., 1983). Such an effect is mimicked by vasopressin administered intravenously, as shown in the present study, suggesting vasopressin administered intravenously may act on the central nervous system.

There is now anatomical evidence linking hypothalamic vasopressin neurones to cardiovascular mid-brain center. Extra-hypothalamic-pituitary vasopressinergic neuronal projections to some of the central cardiovascular regulatory centers have been described. The paraventricular nucleus and supraoptic nucleus, main vasopressin synthesizing nuclei, have been shown to send projections to the locus coeruleus, nucleus tractus solitarius, and anteroventral third ventricular (AV3V) region (Saper et al., 1976; Buijs et al., 1978; Sofroniew, 1980), as well as to receive noradrenergic synapses from these areas (Sawchenko and Swanson, 1981). Vasopressin usually increases the sensitivity of the baroreceptor reflex through its central action in rats. (Izdebska et al., 1982; Imai et al., 1983). Berecek et al. (1982) reported that stimulation of the paraventricular nucleus produced a frequency-dependent bradycardia in rats. However, it has also been reported that stimulation of vasopressin synthesizing nuclei attenuates the bradycardia produced by carotid sinus stimulation, whereas ablation of these areas in-

creases reflex bradycardia in cats (Ciriello and Calaresu, 1980). Intracisternal administration of vasopressin also attenuated the blood-pressure-lowering effect of carotid sinus stimulation in dogs, suggesting suppressed inhibition of central sympathetic vasomotor activity (Brattström and Kalkoff, 1970). Thus, the central action of vasopressin on baroreflex function and central cardiovascular control mechanisms may depend on the species of animal or on its precise anatomical site of action.

The peripheral as well as the central pathways of the baroreceptor reflex could be affected by vasopressin. It has been reported that local application of a variety of drugs to the carotid sinus, or stimulation of sympathetic fibers innervating the sinus wall, results in a fall in blood pressure through stimulation of the afferent limb of the reflex (Kirchheim, 1976). Although the effect of vasopressin on the baroreflex afferent pathway has not yet been studied, the possibility remains that vasopressin may also modulate the baroreflex function via this route.

In the present study, not only AVP but also DDAVP, a vasopressin analogue with potent anti-diuretic activity but with minimal vasoconstrictor action, caused bradycardia and increased baroreflex sensitivity in DI rats. On the other hand, a specific vascular receptor antagonist of vasopressin did not affect the baroreflex sensitivity or heart rate in normal LE rats. The present results suggest that the effect of vasopressin on the baroreflex are mediated through a vasopressin receptor more similar to the vasopressin renal receptor than the vascular receptor. The type of vasopressin receptor mediating the central actions of vasopressin is not yet known. Recently, LeMoal et al. (1981) reported that dPTyr (Me) AVP, one of the specific vascular receptor antagonists of vasopressin, abolished the effect of

vasopressin on active avoidance behavior. However, Weingartner et al. (1981) reported that DDAVP altered aspects of memory function in adult humans. This information suggests that the central effects of vasopressin may be mediated through both types of vasopressin receptor, or a third type of receptor partially influenced by vasopressin and DDAVP. The restoration of baroreflex sensitivity by DDAVP in DI rats may be explained in part, by dual central vasopressin receptors. However, the possibility remains that the central action of vasopressin is mediated through a receptor different from both the renal and vascular receptors of vasopressin. It has recently been shown that some of the activities of vasopressin in the central nervous system can be performed by a smaller fragment of the molecule than is needed or used for peripheral functions (Hoffman et al., 1977; Stewart, 1982).

In the present study, the same dose of AVP and DDAVP increased baroreflex sensitivity and caused bradycardia in DI rats. Although the change in body weight by DDAVP was not detectable, it is known that the antidiuretic potency of DDAVP is 3 times higher than AVP. To exclude the possibility that these effects were due to fluid retention, DI rats were volume expanded with 5% dextrose solution. This, however, did not change the heart rate or baroreflex sensitivity.

The rate of change of the arterial pressure is considered to be an important influence on the baroreflex heart rate response (Kirchheim, 1976). However, there was no difference between LE and DI rats in the pressor effect or the time to attain peak mean arterial pressure following phenylephrine. Thus, it can be concluded that the difference in baroreflex sensitivity between LE and DI rats was not due to differences in the dynamic characteristics of the pressure stimulus.

The heart rate change in response to transient increases in arterial pressure is dependent mainly on the vagus nerve (Warner and Cox, 1962; Scher and Young, 1970; Thames and Kontos, 1970; Pickering et al., 1972). The present study also showed that reflex slowing of the heart in response to transient increases in arterial pressure disappeared almost completely after atropine in both LE and DI rats. If the difference of baroreflex sensitivity between LE and DI rats was partly due to differences in cardiac sympathetic activity, the baroreflex function slope in LE rats should be steeper than that in DI rats when parasympathetic tone was abolished. After atropine treatment, however, the baroreflex function slopes were abolished in both LE and DI rats. Thus, it would appear that the difference in baroreflex sensitivity between LE and DI rats is attributable mainly to differences in the parasympathetic response to a transient increase in blood pressure.

From the results of present experiments, it is obvious that endogenous and exogenous vasopressin can modulate the sensitivity of the baroreflex. Since

AVP and DDAVP decreased heart rate without significant change in arterial pressure, vasopressin may modulate not only the sensitivity but also the set point of the baroreflex (Korner, 1971).

The results obtained in DI rats and the change in baroreflex sensitivity brought about by infusion of vasopressin provide strong evidence that vasopressin may be an important physiological modulator of baroreflex function.

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INDEX TERMS: Baroreflex function • Vasopressin vascular receptor antagonist • Long-Evans rat • Blood pressure • Heart rate • Phenylephrine