Brain vasopressin V₁ receptors contribute to enhanced cardiovascular responses to acute stress in chronically stressed rats and rats with myocardial infarction

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Cudnoch-Jedrzejewska A, Szczerpanska-Sadowska E, Dobruch J, Gomolka R, Puchalska L. Brain vasopressin V₁ receptors contribute to enhanced cardiovascular responses to acute stress in chronically stressed rats and rats with myocardial infarction. Am J Physiol Regul Integr Comp Physiol 298: R672–R680, 2010. First published December 30, 2009; doi:10.1152/ajpregu.00543.2009.—The present study was designed to determine the role of central vasopressin 1 receptors (V₁R) in the regulation of cardiovascular parameters in chronically stressed infarcted rats and sham-operated rats under resting conditions and after exposure to acute alarming stress. The experiments were performed on four groups of conscious sham-operated and four groups of infarcted rats subjected to intraventricular infusion of either vehicle or a V₁R antagonist (V₁RANT). Two groups of infarcted and two groups of sham-operated rats were subjected to mild chronic stressing. Mean arterial blood pressure (MABP) and heart rate (HR) were determined under resting conditions and after exposure to acute stress (air jet). During vehicle infusion, MABP and HR increases in response to acute stress in the infarcted rats not subjected to chronic stress, and in the infarcted and sham-operated chronically stressed rats, were significantly greater than in the sham-operated rats not exposed to chronic stress. However, MABP and HR responses to acute stress in the chronically stressed infarcted rats and chronically stressed sham-operated rats did not differ. V₁RANT abolished differences in cardiovascular responses to acute stress between the experimental groups. Resting cardiovascular parameters were not affected by any of the experimental treatments. It is concluded that chronic stress enhances the pressor and tachycardic responses to acute stress in the sham-operated rats but does not further intensify these responses in infarcted rats. The results provide evidence that central V₁R display involvement in the regulation of cardiovascular responses to acute stress in chronically stressed rats, infarcted rats, and chronically stressed infarcted rats.

vasopressin 1 receptor antagonist; heart failure; tachycardia; depression

A growing number of studies provide evidence that neurogenic stress may provoke sudden cardiovascular complications (7, 15, 29, 36, 44, 49). Recently, a reciprocal relationship between the chronic stress and disorderly regulation of the cardiovascular parameters has been postulated (19–21), although the mechanisms underlying the coupling between stress and cardiovascular events have not been well recognized. Stress engages complex behavioral, neuroendocrine, and cardiovascular responses. Optimum adaptation of the body to stress requires smooth coordination of several effective mechanisms. The central vasopressin system comprising neurons releasing vasopressin (AVP) in the brain and brain vasopressin receptors warrants particular attention as one of the essential stress-activated integrative/regulatory mechanisms.

It has been established that AVP-releasing neurons project to the anterior/preoptic forebrain region which is engaged in initiation and regulation of the behavioral responses to stress (6, 8, 18, 41). Additionally, AVP neurons belong to the hypothalamo-hypophysial-adrenal neuroendocrine stress axis (1, 5, 32, 38). Finally, a number of studies indicate that centrally released AVP plays an important role in the regulation of blood pressure (22, 34, 42). As to vasopressin involvement in the regulation of the cardiovascular system in stress, recent studies provided evidence that centrally released AVP significantly influences the magnitude of the cardiovascular responses to short-lasting alarming stress (12, 16, 40). In addition, it has been demonstrated that, during postinfarction, heart failure-enhanced stimulation of the brain V₁₄ receptors by vasopressin plays an essential role in intensification of the cardiovascular responses to stress (12, 16).

Recent clinical trials and experimental studies strongly suggest that chronic stress and depression may predispose to development of severe cardiovascular complications (9, 17, 22). In view of the above, we decided to determine the role of central vasopressin V₁ receptor (V₁R) in the regulation of cardiovascular parameters in rats with postinfarction heart failure exposed to chronic and subsequently acute stress. Specifically, the present investigation was designed to answer the following questions: 1) whether mild chronic stressing influences resting cardiovascular parameters and the pressor and tachycardic responses to acute alarming stress in infarcted and sham-operated rats and 2) whether chronic blockade of central V₁Rs can significantly modify the effects of chronic stressing on cardiovascular parameters at rest and cardiovascular responses to alarming stress in infarcted and noninfarcted rats. A preliminary report of some of the data included in the present investigation was published in abstract form (43).

METHODS

Animals, Surgical Procedures, and Postsurgical Care

The experiments were performed on male Sprague Dawley rats (SPRD/Mif/Lod) bred in the Department of Animal Breeding of the Medical University of Warsaw. The rats were fed with rodent dry diet containing 0.3% of NaCl and had free access to water. They were maintained on the 12:12-h light-dark rhythm in a room with regulated temperature (range 22–25°C).

The rats entered the study at an age of 8–10 wk. Before the experiments, they were subjected to three surgical procedures (for sequence and plan see Fig. 1A) performed under general anesthesia

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role of brain V1 receptors in chronic stress

A

Chronic procedures

Infarct of sham surgery

Mini pump implantation

Arterial catheter implantation

ICV infusion of vehicle or vehicle + V1ANT

Experiment

8–10 weeks old rat

1 day

14 weeks

chronic stress or resting

B

Course of experiment

ICV infusion of vehicle or vehicle + V1ANT

Observation 10–20 min

Resting

Alarming stress

40 min ICV infusion

MABP and HR measurements

Fig. 1. A: sequence of surgical procedures. B: time course of experiments. HR, heart rate; MABP, mean arterial blood pressure; V1ANT, vasopressin 1 receptor antagonist (deamino-Pen1, O-Me-Tyr2, Arg8)vasopressin.

Myocardial infarction and sham surgery. The rats were randomly divided into two groups. Left coronary artery ligation was performed to evoke myocardial infarction in one group, and sham surgery was performed in the other group. Myocardial infarction was produced using our own modification (16) of the procedure described by Selye et al. (37). In brief, a surgical incision was made between the fourth and the fifth intercostal space, and the heart was exteriorized. Lung ventilation was maintained by frequent administration of air puffs by means of a small rubber balloon connected to the rat’s nose through a plastic tube. The left coronary artery was permanently tied off with a suture thread (Ethicon 6.0). The heart was placed back in the thoracic cavity, and the wound was closed with surgical sutures (Ethicon 4.0). Sham surgery was performed as described above except that the pericardium was only touched with the needle and the coronary artery was not ligated. The postoperative survival rate was equal to 46.6%.

Left ventricle cannula and osmotic minipump implantation for intracerebroventricular infusions. After 7 days that were allowed for recovery from the thoracic surgery, the rats were anesthetized with pentobarbital sodium for implantation of the brain infusion kit together with an osmotic minipump (Alzet, length: 5.1 cm, diameter: 1.4 cm). The rat’s head was placed in the stereotactic apparatus (Kopf) and leveled between the lambda and the bregma. A skin incision was made, and a hole was drilled in the bone at the following stereotactic coordinates: 2 mm lateral to the midsagittal suture and 1.3 mm posterior to bregma. The intraventricular cannula of the brain infusion kit was introduced vertically through the opening in the skull and secured with acrylic cement (Duracryl; Spofa-Dental). The osmotic minipump was inserted under the skin and connected to the cannula of the brain infusion kit, and intracerebroventricular infusion of either 0.9% NaCl (vehicle) or the V1R antagonist ([deamino-Pen1, O-Me-Tyr2, Arg8]vasopressin; Sigma V1880) (2) was started. The antagonist was infused at a rate of 1 μg • rat−1 • 24 h−1. The skin was closed with surgical sutures (Ethicon 4.0). The rat was given penicillin and buprenorphin sulfate and placed in the home cage.

Catheter implantation in the femoral artery. The arterial catheter used for mean arterial blood pressure (MABP) measurements consisted of an intra-arterial part that was a 3.5- to 4.0-cm tube (ID 0.12 mm, OD 0.25 mm) and an external part that was a polyvinyl tube (ID 0.25 mm; OD 0.4 mm; Scientific Commodities). The internal part was inserted in the aorta through the femoral artery so that its end was located 2 cm below the renal arteries. The external part was tunneled under the skin and exteriorized on the neck. The catheter was filled with 0.9% NaCl containing 500 U of heparin/ml and plugged with a stopper. The rat was given penicillin and placed in its home cage. The experiments were performed 24 h after the surgery when the animals fully recovered from the anesthesia and could move without apparent discomfort.

Experimental Groups and Course of the Experiments

Experimental groups. Hemodynamic measurements were successfully completed in 32 infarcted and 30 sham-operated rats that met the experimental requirements (appropriate location of the intraventricular cannula, good performance of the osmotic minipumps). The rats were divided into the following experimental groups: 1) sham-operated rats not exposed to chronic stress and receiving an intracerebroventricular infusion of the vehicle (0.9% NaCl, group 1, n = 8); 2) infarcted rats not exposed to chronic stress and receiving an intracerebroventricular infusion of the vehicle (group 2, n = 9); 3) sham-operated rats not exposed to chronic stress and receiving an intracerebroventricular infusion of the V1R antagonist (V1RANT) (group 3, n = 7); 4) infarcted rats not subjected to chronic stressing and receiving an intracerebroventricular infusion of the V1RANT (group 4, n = 7); 5) sham-operated chronically stressed rats receiving intracerebroventricular infusion of vehicle (group 5, n = 8); 6) infarcted chronically stressed rats receiving an intracerebroventricular infusion of the vehicle (group 6, n = 8); 7) sham-operated chronically stressed rats receiving an intracerebroventricular infusion of the V1RANT (group 7, n = 7); and 8) infarcted chronically stressed rats receiving an intracerebroventricular infusion of the V1RANT (group 8, n = 8).

The V1 receptor antagonist used in the present study ([deamino-Pen1, O-Me-Tyr2, Arg8]vasopressin) blocks mainly V1 vasopressin receptors (antivasopressor pA2 = 7.96 ± 0.05) and has only weak oxytocin receptor antagonist properties (in vitro pA2 = 7.61 ± 0.14). In addition, it displays weak V2 receptor agonist activity (antidiuretic potency 3.5 ± 0.5 U/mg) (2). The experimental design is shown in Fig. 1B.

Chronic stressing. Chronic stressing was performed according to a modification of the program described by Grippo et al. (25). The stressing program used by us consisted of five sessions (1 session/day, 5 sessions/wk) repeated within 4 wk. At each session, the rat was subjected to one of the following procedures: 1) exposure to stroboscopic lamp flashes (300 flashes/min for 5 h), 2) placing the rat’s cage in an oblique position at an angle of 40° for 6 h/day, 3) a visit of another rat in the home cage of the experimental rat; the rats were separated by a transparent partition at the visit, 4) water deprivation for 18 h followed by access to an empty bottle for 6 h, or 5) placement in a new smaller cage (30 × 30 × 30 cm) where the rat was exposed to an alien smell (deodorant placed in a perforated box). After 2 days of rest, the rats underwent the next stressing session, but the sequence of the stressing procedures was altered.

Alarming stress. Air jet stress was used to evoke short-term alarming stress. The air jet was blown on the top of the rat’s head for 1 s via a laboratory-made device (16). The device consists of a tank with compressed air and a pressure-reducing unit connected through a plastic tube (ID 3.0 mm) to a funnel (ID 41.5 mm). The funnel was held above the rat’s head at a distance of 1.5–2.0 cm, and the pressure of the air delivered from the funnel on the rat’s head was 1.5 atm.
During application of the air jet stress, the rat was not restrained.

Hemodynamic measurements. Hemodynamic measurements are presented in Fig. 1B. First, the arterial catheter was connected to a blood pressure recording unit (see below). After 30 min allowed for adaptation, MABP and heart rate (HR) were recorded continuously for 40 min under resting conditions and for the next 10 min after application of an alarming stressor (air jet). To determine the resting MABP and HR, the data collected during 40 min of the resting period were averaged. The maximum increases of MABP and HR evoked by the air jet stress were determined by subtracting the resting MABP and HR (the MABP and HR values immediately preceding stressor application) from the maximum values of these parameters; found in the first 5 s after stressor application. The latency to the maximum increases in MABP and HR and the duration of the pressor and tachycardic responses were also determined. The rats did not have access to food and water during the experiment to avoid accidental fluctuations of MABP and HR associated with the ingestive behavior.

Measurements of the Cardiovascular Parameters

MABP and HR. During the experiment, the external part of the catheter was connected with a blood pressure and HR recording system (MP 100; Biopac, Santa Barbara, CA) collecting the systolic, diastolic, and pulse pressure data and calculating the MABP and the HR. The system determines MABP as an area under the arterial pressure curve divided by the cardiac cycle duration and calculates the HR (beats/min) from the number of the systolic pressure peaks. The resting MABP and HR were determined by averaging the data collected in the 40 min of rest.

Measurements of end-diastolic ventricular pressure. After completion of the experiment, the rat was anesthetized, and a thin catheter (ID 0.5 mm, OD 0.8 mm; Dural Plastics and Engineering, Auburn, Australia) was inserted in the left cardiac ventricle via the carotid artery. Pressure was recorded with a blood pressure recording system (MP100; Biopac).

Postmortem Examination

After the experiments, the rats were killed by an overdose of pentobarbital (pentobarbital sodium 10 mg/100 g body wt ip). The heart was excised from the thorax, and the thoracic cavity was inspected for the presence of edema fluid. Both ventricles were weighed. The infarction surface was determined planimetrically (30) with some modifications (16). In brief, the wall of the left ventricle (including septum) was separated from the right ventricle along the longitudinal axis and placed flat on a transparent paper sheet. Circumferences of the internal and external ventricle surface were outlined. The infarction surface expressed as the number of square millimeters was estimated on both sides of the ventricle and averaged. The mean of the two measurements was calculated, and the infarction surface was expressed as a percentage of the left ventricle wall surface, including the septum. The rats in which the infarction surface was <25% of the left ventricular surface were excluded from further evaluation, since we found in our previous studies that the rats with myocardial infarction of <25% of the left ventricular surface did not always manifest heart failure symptoms, such as a significant increase in left ventricular end-diastolic pressure and the presence of typical postinfarction fibrosis in histological examination (12, 13, 16). In the present study, histological verification was also performed in some of the rats. For this purpose, heart fragments were placed in 10% formaldehyde solution. Fixed tissue blocks were paraffin-embedded and sectioned at 4 μm. The slices were processed by hematoxilin and eosin staining for routine morphological examination. In each case, the histological examination confirmed the presence of an infarction scar in the heart region that was found to be infarcted on prior visual inspection. A typical example of a postinfarction scar is shown in Fig. 2.

Position of the guide tube in the lateral ventricle. The osmotic minipump was disconnected and removed to inspect if the infusion had been performed successfully.

Evans Blue was injected in the lateral ventricle through the intracerebroventricular cannula of the brain infusion kit to determine whether the cannula communicated with the ventricular system. The brain was isolated, and sagittal sections were made to inspect visually the ventricular system for the presence of the dye. All rats in the experimental groups had proper location of the intracerebroventricular cannula.

Statistical Analysis

The Statistica software (version 7) was used for statistical analysis of the data using the indications recommended by Curran-Everett and Benos (14) and Ludbrook (33). Two way-ANOVA was applied to estimate the significance of differences between the resting MABP and HR values found after 4 wk of intracerebro-
ventricular infarctions and to determine the significance of differences between the maximum increases in MABP and HR evoked by air-jet stress in the individual experiment series. The horizontal and vertical multiple pair-wise comparisons were made using the post hoc Tukey test. The differences were considered significant if \( P < 0.05 \). All values and Figs. 1–5 presented in the text are means ± SE.

RESULTS

Measurements Under Resting Conditions

The mean body weights of the rats assigned to the individual experimental groups did not differ significantly (group 1: 328 ± 9 g; group 2: 330 ± 5 g; group 3: 334 ± 9 g; group 4: 316 ± 6 g; group 5: 341 ± 8 g; group 6: 326 ± 8 g; group 7: 329 ± 7 g; and group 8: 334 ± 10 g).

Differences between the infarcted and sham-operated rats. The infarction surface was similar in all groups of infarcted rats (group 2: 40.33 ± 2.45%; group 4: 37.29 ± 3.21%; group 6: 40.44 ± 2.42%; and group 8: 39.56 ± 2.77%). Left ventricular end-diastolic pressure was significantly higher in the infarcted rats than in the sham-operated rats [overall analysis of variance: \( F(1,60) = 468.23; P < 0.001 \); intergroup analysis of variance: \( F(7,54) = 63.750; P < 0.001 \) (Fig. 3A). Left ventricle weight was significantly greater in the infarcted rats than in the sham-operated rats \( F(1,60) = 6.612; P < 0.05 \); however, there were no significant differences between the individual experimental groups of the infarcted and sham-operated rats (Fig. 3B). The right ventricle weight in the infarcted rats and sham-operated rats did not differ (Fig. 3C). A significant difference was found between the MABP values in the infarcted and sham-operated rats \( F(1,60) = 55.980 \) and between the individual groups of the infarcted and sham-operated rats \( F(7,54) = 13, 467; P < 0.001 \) (Fig. 4A). The HR was similar in all experimental groups (Fig. 4B).

Effects of chronic stressing. Resting MABP was significantly higher in the chronically stressed rats than in the rats that were not exposed to chronic stress \( F(1,60) = 4.663; P < 0.03 \). Comparison of the individual experimental groups revealed significant difference between the resting MABP of the infarcted chronically stressed group receiving vehicle and the MABP of the infarcted group receiving the V1RANT and not exposed to chronic stressing (Fig. 4A). The HR, left ventricular end-diastolic pressure, left ventricle weight, and right ventricle weight in the chronically stressed rats were not significantly different from the corresponding values found in the rats not exposed to chronic stress (Figs. 3 and 4B).

Effects of chronic blockade of V1Rs. Resting MABP was significantly lower in the whole group of rats receiving the V1RANT infusion than in the whole group of rats infused with the vehicle \( F(1,60) = 4.351; P < 0.04 \) (Fig. 4). Nevertheless, comparison between the individual groups revealed that a significant difference was present only between the group of infarcted rats not exposed to chronic stress but infused with the V1RANT and the group of chronically stressed infarcted rats receiving the vehicle (see above and Fig. 4A). The HR, left ventricular end-diastolic pressure, and weights of the left and right ventricles of the rats infused with the V1RANT and those infused with the vehicle did not differ (Figs. 3 and 4B).

Cardiovascular Responses to the Alarming Stress in the Infarcted and Sham-Operated Rats Exposed or Not Exposed to Chronic Stress

Maximum MABP responses to alarming stress. The maximum increases in MABP in response to alarming stress were significantly greater in the infarcted rats than in the sham-operated rats \( F(1,60) = 7.174; P < 0.01 \). As revealed by detailed analysis of variance, significant differences were also present between the individual groups of the infarcted rats and sham-operated rats \( F(7,54) = 9.407; P < 0.001 \). Specifically, significant difference was found between the group of sham-operated rats and the group of infarcted rats that received the vehicle and were not exposed to chronic stress (post hoc Tukey test, \( P < 0.001 \); Fig. 5A). The Tukey test also revealed a significant difference between the maximumpressor responses to air jet stress between the sham-operated group receiving the vehicle and the chronically stressed sham-operated group infused with the vehicle \( P < 0.05 \); Fig. 5A). No significant
difference was found between $\Delta MABP_{\text{max}}$ evoked by the air jet stress in the group of infarcted, nonchronically stressed rats receiving the vehicle and the group of infarcted chronically stressed rats receiving the $V_1\text{RANT}$ ($P < 0.001$), and 4) the group of infarcted, nonchronically stressed rats infused with the vehicle and the group of infarcted chronically stressed rats infused with the $V_1\text{RANT}$ ($P < 0.001$). In all of these groups, intracerebroventricular administration of the $V_1\text{RANT}$ reduced $\Delta MABP_{\text{max}}$ responses to the air jet stress to a level that was not significantly different from that found in the sham-operated rats, not exposed to chronic stress. No significant differences in $\Delta MABP_{\text{max}}$ were found between the individual groups of rats receiving the $V_1\text{RANT}$ (Fig. 5A).

Maximum HR responses to the alarming stress. Significant differences in the maximum air jet stress-induced HR increases were found between the rats subjected to the central blockade of the $V_1\text{R}$ and those receiving the vehicle [$F(1,60) = 23.692; P < 0.001$]. The overall analysis of variance revealed significant differences in $\Delta HR_{\text{max}}$ between the individual experimental groups [$F(7,54) = 5.198; P < 0.001$]. As shown in Fig. 5B, the infarcted rats receiving the vehicle and not exposed to Fig. 5. Mean increases of MABP ($A$) and HR ($B$) from baseline evoked by air jet stress in the sham-operated and infarcted rats subjected or not subjected to chronic stressing and to icv infusion of vehicle or V1ANT. Means $\pm$ SE are shown. Significant differences between the experimental groups: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.0001$.
chronic stress responded with a significantly greater $\Delta \text{HR}_{\text{max}}$ to the air jet stress than the sham-operated rats receiving the vehicle and not exposed to chronic stress ($P < 0.02$; Fig. 5B). However, there was no significant difference between the infarcted chronically stressed rats receiving the vehicle and the sham-operated chronically stressed rats receiving the vehicle (Fig. 5B). The $\text{V}_{1}\text{RANT}$ infusion significantly altered the magnitude of $\Delta \text{HR}_{\text{max}}$ responses to the air jet stress (Fig. 5B). In particular, significant differences were found between 1) the group of infarcted rats not exposed to chronic stress and infused with the vehicle and the group of infarcted, nonchronically stressed rats infused with the $\text{V}_{1}\text{RANT}$ ($P < 0.001$), 2) the group of chronically stressed infarcted rats receiving the vehicle and the group of chronically stressed infarcted rats receiving the $\text{V}_{1}\text{RANT}$ ($P < 0.001$), and 3) the group of chronically stressed sham-operated rats receiving the vehicle and the group of chronically stressed, sham-operated rats receiving the $\text{V}_{1}\text{RANT}$ ($P < 0.05$). In all of the above groups, intracerebroventricular administration of the $\text{V}_{1}\text{RANT}$ reduced $\Delta \text{HR}_{\text{max}}$ responses to the air jet stress to a similar level as in the sham-operated rats not exposed to chronic stress. No significant differences in $\Delta \text{HR}_{\text{max}}$ were found between the individual groups of rats receiving $\text{V}_{1}\text{RANT}$ (Fig. 5B).

The duration of MABP and HR increases evoked by alarming stress was similar in all experimental groups. The latency to the tachycardic response was significantly shorter in the whole population of infarcted rats than in the whole population of sham-operated rats [$F(1,60) = 5.996; P < 0.05$], but no significant differences were found between the individual experimental groups.

**DISCUSSION**

The present investigation reveals a new role of central $\text{V}_{1}\text{Rs}$ in the regulation of cardiovascular parameters in chronically stressed infarcted rats and sham-operated rats. The evidence is provided that chronic stressing significantly intensifies the cardiovascular responses to alarming stress in sham-operated rats. In addition, the study confirms the results of the previous reports that cardiovascular responses to stress are augmented during postinfarct heart failure (12, 16, 48). At the same time, the present study shows that the chronic stressing does not further intensify the cardiovascular responses to alarming stress in the infarcted rats. The major finding of the present investigation is the demonstration that the brain vasopressin $\text{V}_{1}\text{Rs}$ are involved in the augmentation of the cardiovascular responses to alarming stress in chronically stressed infarcted rats and sham-operated rats. We discuss the present results in relevance to 1) the impact of chronic stressing on the regulation of cardiovascular parameters under resting conditions and under alarming stress in infarcted and sham-operated rats, 2) the role of the brain $\text{V}_{1}\text{Rs}$ in generation of exaggerated cardiovascular responses to alarming stress in chronically stressed infarcted rats and sham-operated rats, 3) the putative causes of the lack of differences between the magnitudes of cardiovascular responses to alarming stress in infarcted and sham-operated rats exposed to chronic stress, and 4) the possible implications of our findings for the treatment of negative consequences of chronic stress and depression.

**Effect of Chronic Stressing on Cardiovascular Parameters at Rest and Under Alarming Stress**

In accordance with the data reported by other authors (48), we found that the infarcted rats had significantly lower MABP than the sham-operated rats. The comparison of resting MABP and HR in the rats exposed and not exposed to chronic stress revealed that the procedure of stressing applied in the present investigation did not have a significant impact on the resting cardiovascular parameters. The infarcted rats manifested significantly lower resting MABP and higher left ventricular end-diastolic pressure whether or not they were chronically stressed or whether they were subjected to the blockade of the central $\text{V}_{1}\text{Rs}$. Grippo et al. (25) reported that chronic stressing caused elevation of the resting HR in rats. Most likely, the discrepancy between the results of our study and that of Grippo et al. (25) is attributable to the differences in the chronic stressing programs. Namely, in our study, the rats were stressed only for 5 days/wk instead of 7 days/wk. The stress load was reduced because our preliminary experiments suggested that the formerly used stressing program (25) might be poorly tolerated by the infarcted rats as manifested by their oversensitivity to anesthesia, increased postsurgical mortality, and restlessness during the experiments.

In agreement with previous studies of Grippo et al. (25), our investigation demonstrates that chronically stressed sham-operated rats respond with significantly greater increases in MABP and HR to the air jet alarming stress. In the study by Grippo et al. (25), the air jet stress was applied for 3 min while in our investigation it was limited to 1 s. Nevertheless, both studies indicate a significant intensifying effect of chronic stressing on the magnitude of the cardiovascular responses to the alarming stressors. In addition, our study reveals that, even if chronic stressing is too weak to affect significantly the resting cardiovascular parameters, it may cause a significant increase in responsiveness of the cardiovascular neurons to the alarming stressors. This phenomenon may account for a greater number of cardiovascular events reported in the subjects experiencing various kinds of chronic stressing (7).

**Involvement of $\text{V}_{1}\text{Rs}$ in Enhancement of Pressor and Tachycardic Responses to the Alarming Stress in Chronically Stressed Rats**

It has been long recognized that corticotropin-releasing hormone (CRH) and vasopressin exert a combined stimulatory effect on the adrenocorticotrophic hormone-cortisol endocrine axis and are essential for its activation in stress (1, 3, 11, 24). There is also evidence that the hypothalamic-pituitary-adrenal axis is stimulated during chronic mild stressing. For instance, the study by Grippo et al. (24) revealed that chronic mild stressing causes a significant increase in corticosterone concentration. In addition, chronic mild stressing was found to elevate the expression of CRH mRNA in the hypothalamus (17). Altogether these findings suggest that chronic stressing activates the whole hypothalamic-pituitary-adrenal axis. Previous studies have demonstrated that prolonged stress exerts a sensitizing effect on the neuroendocrine system. For instance, Bartanusz et al. (5) provided evidence that the repeated immobilization stress resulted in an enhanced production of AVP in the paraventricular nucleus. Recently, Yoshii et al. (47) reported that severe prolonged stress caused significant changes
in AVP mRNA expression and altered immunoreactive optical density of AVP-containing neurons in the supraoptic nucleus. The changes overlasted the stressing period and had an influence on AVP content in dendrites during subsequent short-lasting stress (forced swimming). Recently, it has been shown that vasopressin, and especially its action via the V₁b receptors, plays a significant role in prolonged activation of the hypothalamic-pituitary-adrenal axis during chronic stressing and exposure to novel stimuli (1, 32, 38). In the present study, V₁R blockade was deliberately applied simultaneously with chronic stressing to avoid the effects of prolonged stimulation of vasopressinergic neurons by stress. The available evidence suggests that both V₁a and V₁b receptors are involved in behavioral and depression-triggering effects of vasopressin (6, 18, 23) and in the regulation of cardiovascular parameters in acute stress (12, 16, 40). In the present study, we decided to use the combined V₁a/V₁b receptor antagonist to determine the effect of simultaneous blockade of both types of these receptors during chronic stressing. The results reveal that the chronically stressed rats subjected to long intracerebroventricular infusion of the combined V₁a/V₁b receptor antagonist manifest significantly lower pressor and tachycardic responses to alarming stress than rats receiving intracerebroventricular infusion of the vehicle. Thus the central V₁R play an essential role in enhancement of cardiovascular responses to a novel stress after chronic exposure to the mild stressing. The mechanism of this phenomenon, and the locus of the sensitizing effect of vasopressin through V₁R cannot be determined at present. Vasopressin-containing neuronal fibers and V₁Rs have been found both in the regions of the brain stem comprising the cardiovascular neurons and in the forebrain structures responsible for behavioral and neuroendocrine responses to stress (4, 27). Thus vasopressin may enhance the cardiovascular responses to stress either directly by activation of the V₁R located on the cardiovascular neurons or indirectly by stimulating those neurons in these forebrain structures that are involved in the regulation of other functions such as recognition, memorizing, and emotional analysis of the stressing stimuli. Signals from the latter neurons can be subsequently transmitted to the cardiovascular neurons.

Although the V₁RANT used in the present study ([deamino-Pen¹,O-Me-Tyr²,Arg³]vasopressin) has mainly V₁R antagonistic properties (antivasopressor pA₂ = 7.96 ± 0.05), it is also a weak oxytocin receptor antagonist (in vitro pA₂ = 7.61 ± 0.14) and a weak V₂ receptor agonist (antidiuretic potency 3.5 ± 0.5 U/mg) (2). In one of the previous studies, we have found that oxytocin receptors in the brain were engaged in attenuation of cardiovascular responses to the alarming stress (45). Therefore, it is possible that simultaneous interference of V₁R antagonist with the oxytocin receptors could modify to some extent (i.e., to reduce) the effects of blockade of V₁R. In the study of Stojicic et al. (40), blockade of central V₁ receptors shortened the duration of cardiovascular response to alarming stress. However, it should be noted that V₂ agonistic properties of [deamino-Pen¹,O-Me-Tyr²,Arg³]vasopressin are very weak and most likely could not have any significant influence on the results of the present investigation. Finally, it should be emphasized that, although the present results give strong evidence for involvement of the brain vasopressin system in enhancement of cardiovascular responses to alarming stress in chronically stressed rats, it cannot be excluded that some other factors, apart from vasopressin, can participate in determining the magnitude of these responses. In particular, our preliminary data (43) suggest also the involvement of ANG II.

The Role of V₁Rs in Regulation of Cardiovascular Responses to Alarming Stress in Infarcted Rats Exposed and Not Exposed to Chronic Stress

In accordance with previous reports (12, 16, 48), the rats with postinfarction heart failure manifested significantly greater pressor and tachycardic responses to stress than the sham-operated rats. Surprisingly, the chronically stressed infarcted rats and the chronically stressed sham-operated rats responded with similar enhancement of cardiovascular responses to alarming stress. Currently, we can propose two mechanisms to explain this finding. First, it may be hypothesized that the stimuli that are chronically generated in the infarcted heart or are associated with the postinfarction heart failure, such as pain, hypoxia, and anxiety, may act as chronic stressors. It may be hypothecized that they are converging on the same neuronal pathways that are activated by the stimuli generated by programmed chronic stressing. It is likely that these factors are potent enough to exert the maximum effect. The second possibility is that the lack of a significant difference in the magnitude of cardiovascular responses to alarming stress between the infarcted rats exposed and not exposed to chronic stress may result from the inability of the failing heart to further increase the cardiac output (the “failing heart” effect). Significant increases in end-diastolic ventricular pressure indicate that left ventricular function was significantly impaired in the infarcted rats used in the present investigation; however, the failing heart effect does not fully explain why the cardioacceleratory response to stress is not augmented by chronic stressing in infarcted rats.

In conclusion, the present study demonstrates that chronic stressing does not appreciably influence the resting cardiovascular parameters, although it significantly intensifies the pressor and tachycardic responses to concomitant acute stress in sham-operated but not in infarcted rats. Furthermore, the study reveals that stimulation of brain V₁Rs is involved in potentiation of the cardiovascular responses to acute stress in chronically stressed rats, infarcted rats, and chronically stressed infarcted rats.

Perspectives and Significance

Results of the present investigation strongly suggest that chronic stressing increases vasopressinergic system activity that in turn results in an increased sensitivity of the cardiovascular neurons to the new stressors and in potentiation of the pressor and tachycardic responses to alarming stress. A question arises about the pathophysiological significance of the enhanced activation of the brain vasopressinergic system during chronic stressing and about the intensification of cardiovascular responses to the novel stressors in chronically stressed subjects. It may be hypothesized that these effects, together with the analgesic effect of vasopressin (46), could have some positive aspects in healthy subjects, since they might improve the anticipatory adaptation of the cardiovascular system to the fight and flight challenges. However, in chronic stressing or postinfarction heart failure, the positive effects of vasopressin on the cardiovascular system may be dominated by its negative
(anxiogenic, depressive, hypertensive) actions. Considering the various aspects of vasopressin action via V1R, it appears that V1R antagonists may prove to be particularly useful as compounds preventing acute cardiovascular complications in patients with cardiovascular disorders and concomitantly exposed to the chronic stressing. Future studies are necessary to determine whether peripheral administration of V1R antagonists could efficiently reduce cardiovascular responses to stress in chronically stressed or infarcted rats and to clarify whether the introduction of these compounds after the commencement of chronic stressing would also be effective.

A significantly greater frequency of life-threatening cardiovascular events was also reported in patients with depression (9, 10, 19–21, 31, 35). Because the procedure of chronic mild stressing used in the present study appeared to be effective in provoking depression symptoms in the rat (25, 26, 28), it is likely that the results of the present study may also have positive implications for the development of improved treatment or prevention of cardiovascular complications in depressive patients. Positive effects of a nonpeptide V1b antagonist in the treatment of depression-like symptoms in the rats have previously been reported by Stemmelin et al. (39).

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DISCLOSURES

No conflicts of interest are declared by the authors.

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