Forebrain-mediated adaptations to myocardial infarction in the rat

JOSEPH FRANCIS,2 SHUN-GUANG WEI,2 ROBERT M. WEISS,1,2 TERRY BELTZ,3 ALAN KIM JOHNSON,3 AND ROBERT B. FELDER1,2

1Department of Veterans Affairs Medical Center and Departments of 2Internal Medicine and 3Psychology, University of Iowa, Iowa City, Iowa 52242

Received 5 June 2001; accepted in final form 7 January 2002

Francis, Joseph, Shun-Guang Wei, Robert M. Weiss, Terry Beltz, Alan Kim Johnson, and Robert B. Felder. Forebrain-mediated adaptations to myocardial infarction in the rat. Am J Physiol Heart Circ Physiol 282: H1898–H1906, 2002; 10.1152/ajpheart.00488.2001.—Recent studies suggest that the forebrain contributes to the circulatory derangements leading to heart failure after myocardial infarction. We tested that hypothesis by examining the effect of myocardial infarction (MI) or sham MI (MI-s) on neurohumoral regulation in rats with prior anteroventral (AV) third ventricle lesion (AV3V-x) or sham lesion (AV3V-s). AV3V-s/MI rats had higher sodium intake, lower urine volume, and lower urinary sodium excretion than AV3V-s/MI-s rats. AV3V-x/MI rats had lower sodium intake and higher urine volume than AV3V-s/MI or AV3V-s/MI-s rats and urinary sodium excretion comparable to AV3V-s/MI-s rats. AV3V-x had no effect on baseline plasma renin activity (PRA). One week after MI, PRA had increased in AV3V-s but decreased in AV3V-x rats. AV3V-x reduced renal sympathetic nerve activity in MI and MI-s rats. AV3V-x improved baroreflex function in MI rats but diminished it in MI-s rats. Survival beyond 2 wk was lower in the AV3V-x/MI rats than in all other groups. These results confirm a critical role for the forebrain in the neurohumoral adjustments to MI.

anteroventral third ventricle; baroreflex; sympathetic drive; volume regulation; plasma renin activity

CONGESTIVE HEART FAILURE (HF) is characterized by fluid accumulation, increased sympathetic drive, and altered baroreflex regulation (31). These homeostatic abnormalities can be mimicked in normal rats by activating forebrain angiotensin (ANG) type 1 receptors. Blood-borne ANG II, acting via ANG type 1 receptors in the circumventricular organs (CVOs) surrounding the anterior third ventricle, stimulates thirst and sodium appetite, initiates the release of the antidiuretic and vasoconstrictor peptide arginine vasopressin, contributes to sympathetic activation (7, 17, 24), and modulates baroreflex regulation (2). These influences of blood-borne ANG II can be blocked by a lesion in the anteroventral (AV) third ventricle (AV3V) region (7), which interrupts the descending pathways from forebrain CVOs and from median preoptic nucleus to critical hypothalamic effector neurons.

In the rat model of ischemia-induced HF, the activity within one of these effector sites, the paraventricular nucleus of the hypothalamus, is increased, suggesting that this region of the brain is either contributing to or reacting to the development of the HF syndrome. Paraventricular nucleus neurons respond to ascending inputs from brain stem regions that process afferent signals from chemosensitive and mechanosensitive receptors in the cardiovascular system and to descending inputs from CVO neurons stimulated by circulating neuropeptides like ANG II. In HF, both the ascending and the descending systems impinging on the forebrain are altered. The present study examined the effect of a lesion in the AV3V region on neurohumoral mechanisms leading to HF after myocardial infarction (MI) in rats. The hypothesis was that activation of forebrain regions regulating fluid balance and sympathetic drive contributes to the progression of heart failure after MI. In normal rats, the AV3V lesion interrupts the effects of blood-borne ANG II to induce sodium appetite, thirst, and sympathetically mediated increases in arterial pressure (7), and in experimental models it prevents the development of hypertension (6).

The results demonstrate that an AV3V lesion has both beneficial and detrimental effects after acute MI. It prevents the abnormalities of volume and sympathetic regulation early after MI, but eventuates in reduced survival as early as 3 wk after MI. Instead of blocking the effects of an overly active renin-angiotensin-aldosterone system (RAAS), it prevents activation of RAAS post-MI. These results call attention to the importance of forebrain mechanisms in mediating the neurohumoral changes that lead to HF after MI. A more precise delineation of the specific mechanisms involved will require further study.

Address for reprint requests and other correspondence: R. B. Felder, Univ. of Iowa College of Medicine, E318-GH, 200 Hawkins Dr., Iowa City, IA 52242 (E-mail: robert-felder@uiowa.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpheart.org
MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (3–4 mo old), weighing 300–325 g, were obtained from Harlan Sprague Dawley (Indianapolis, IN). They were housed in temperature-controlled (23 ± 2°C) and light-controlled (lights on between 0500 and 1700 h) animal quarters and were provided with rat chow ad libitum. The experimental procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

Protocols

Rats underwent AV3V lesion (AV3V-x) or sham AV3V lesion (AV3V-s) and 6 wk later underwent coronary artery ligation (CL) to induce MI or sham CL (MI-s). There were two experimental groups. One group was kept in metabolic cages. Two or three weeks after MI or MI-s, these animals were reanesthetized briefly to implant renal nerve recording electrodes. Four hours after recovery from anesthesia, baseline recordings of sympathetic nerve activity and arterial pressure were obtained and baroreflex testing was performed. These animals then underwent terminal anesthesia for measurements of left ventricular (LV) end-diastolic pressure.

Surgical Preparations

All surgical procedures were performed using sterile techniques, and animals were treated for postoperative pain with subcutaneous injection of buprenorphine (0.03 mg/kg, Q 12 h ×2, then as needed).

AV3V lesioning. AV3V lesions were performed using methods previously described (3, 4, 8). Rats were anesthetized with an Equithesin-like anesthetic cocktail (0.97 g of pentobarbital sodium and 4.25 g of chloral hydrate per 100 ml distilled water; 0.33 ml/100 g). The animals were secured in a stereotaxic apparatus (Kopf Instruments; Tujunga, CA) with the skull leveled between the bregma and lambda. A monopolar electrode made of 24-gauge nichrome wire (Driver-Harris; Harrison, NJ), insulated except at the tip, was placed in the AV3V region using stereotaxic coordinates (midline; 0.3 mm caudal to bregma; 7.5 mm ventral to dura). Anodal current (2–3 mA for 25–30 s) was passed between the monopolar electrode in the AV3V region and a reference electrode placed in the rectum. In AV3V-s, the brain electrode was placed 1.0 mm above the target coordinates and no current was passed.

AV3V-x were considered successful if the rats were adipsic for the first 24 to 48 h. The AV3V-x animals were given 10% sucrose solution to prevent dehydration, and the concentration of sucrose was gradually tapered over a period of 2 wk until they began drinking regular water. At the end of the study, the brains were examined to verify the location of lesions.

Jugular catheterization. After the animals were placed under ketamine and xylazine anesthesia (90 and 10 mg/kg ip, respectively), a midline cervical incision was made and the jugular vein was isolated by blunt dissection. A Silastic catheter (0.025-in. ID and 0.047-in. OD) (Dow Corning; Midland, MI) was inserted into the vein and held in position by sutures. The free end of the catheter was externalized to the base of the skull. The catheter was flushed every other day for the first week and then every day with a mixture of polyvinyl pyrrolidone, bacteriostatic saline, and heparin sodium (100 U/ml), and was sealed with a piece of blunt steel tubing to prevent clogging.

Induction of MI. Six weeks after AV3V surgery, MI was induced by coronary artery ligation. Rats were anesthetized with ketamine and xylazine (90 and 10 mg/kg ip, respectively), endotracheally intubated, and mechanically ventilated (room air, tidal volume 2.5 ml, 50–55 breaths/min). Under sterile conditions, a left thoracotomy was performed to expose the heart. The pericardium was opened and the heart was exteriorized. The left anterior descending coronary artery was ligated between the pulmonary outflow tract and the left atrium with a 6-0 silk suture that passed through the superficial layers of myocardium. The heart was returned to the chest cavity, the lungs were reinflated, and the chest incision was closed. MI-s rats were prepared in the same manner but did not undergo coronary artery ligation. After completion of the surgical procedures, rats were removed from the ventilator and the endotracheal tube was removed. Post-surgical animals were given an intramuscular injection of benzathine penicillin (30,000 units) and four intramuscular injections of lidocaine (2 mg, Q 2 h).

Successful creation of MI was confirmed by echocardiography performed within 24 h after operation on MI and MI-s rats and by visual inspection of the heart at the conclusion of the study.

Implantation of sympathetic nerve recording electrodes. While under pentobarbital anesthesia (50 mg/kg ip), the left femoral artery and vein were cannulated, and the catheters were tunneled to the back of the neck. The left kidney was exposed via a flank incision. One of the nerves to the left kidney was dissected free from surrounding tissue and placed on bipolar silver wire recording electrodes. When an optimal signal-to-noise ratio was achieved, the electrode and the renal nerve were covered with polyvinylsiloxane (President light, Coltene), and the electrodes were sutured to back muscle and then tunneled to the back of neck.

Data Acquisition

Echocardiographic assessment of LV function. Echocardiographic assessment was performed as previously described (12, 13). Echocardiography was performed using an Acuson (Mountain View, CA) Sequoia clinical imager fitted with an 8-MHz sector-array probe, which generates two-dimensional images at a rate of ~100/s. Animals were sedated with ketamine (25 mg/kg ip) to facilitate positioning for echocardiographic study. The anterior chest was shaved and prewarmed acoustic coupling gel was applied. The animal was positioned in the left lateral recumbent position to optimize the windows for echocardiography. The probe was applied gently to the chest. Short-axis images were acquired parallel to the mitral valve plane to obtain the largest cross-sectional image of the left ventricle, which does not contain the mitral valve. Long-axis views were obtained perpendicular to the mitral valve plane and were deemed optimal when the diastolic apex-to-base length was longest and when both mitral and aortic valves were visible. Pulse-wave Doppler tracings were obtained with gates placed so as to interrogate mitral inflow. Images were written to a magnetooptical disk for subsequent off-line analysis.

AJP-Heart Circ Physiol • VOL 282 • MAY 2002 • www.ajpheart.org
proprietary software, which was developed specifically for this purpose by the vendor (Acuson). Heart rate (HR) was determined from the Doppler tracings. LV end-diastolic volume (LVEDV), LV end-systolic volume, LV ejection fraction (LVEF), stroke volume, and LV mass (M) were computed using the area length method, which has been validated in rodents (15) and humans (27). The portion of the LV that displayed akinesis was planimetered electronically and was expressed as a percentage of the total LV silhouette to estimate the size of the ischemic zone (%). Only animals with large infarctions (% ischemic zone ≥ 35%; range 36–66%) were used in the study.

**Metabolic cage assessment of fluid balance.** Ingestion of food, water, and 1.8% NaCl, body weight, urine volume, and urinary sodium content were measured over two consecutive 24-h periods each week, and an average daily value for each variable was reported for that time point. Urine sodium was measured using a sodium and potassium analyzer (NOVA Biomedical; Waltham, MA).

**Papillary muscle measurements.** A blood sample was collected at baseline 2 days before CL or sham CL and then at day 7 and day 14 for the measurement of PRA. Blood samples were centrifuged at 4°C, and the plasma samples were separated and stored at −70°C until assayed using radioimmunoassay. After each collection, blood cells were reconstituted with the same volume of bacteriostatic heparin in 0.9% saline and reinfused. ANG I was measured and expressed as PRA with the use of an ANG I radioimmunoassay kit (NEN Life Science; Boston, MA).

**Renal sympathetic nerve recording and baroreflex testing.** Sympathetic nerve recordings were made in the conscious, freely mobile state 4 h after recovery from anesthesia to implant bipolar electrodes on the renal nerve. The externalized recording electrodes were connected to a Grass P511 AC amplifier to record renal sympathetic nerve activity (RSNA). RSNA was then passed to a Paynter filter (20-ms time constant; Bak Electronics; Germantown, MD) to rectify and integrate the raw signal. The externalized femoral artery catheter was connected to a strain gauge transducer to record mean arterial pressure (MAP). HR was derived by computer analysis of the raw signal. The externalized femoral artery catheter was positioned at a site within the left ventricle at which LV pressure could be accurately recorded (i.e., the onset of the rapid rise in LV pressure after atrial contraction could be observed) and LV systolic pressure was not higher than aortic pressure on entering the LV chamber. Pressure was recorded continuously while the cannula was positioned at a site within the left ventricle at which LV pressure could be accurately recorded (i.e., the onset of the rapid rise in LV pressure after atrial contraction could be observed) and LV systolic pressure was not higher than aortic pressure on entering the LV (i.e., there was no evidence of ventricular outflow obstruction by the cannula). LV pressure was then recorded for an interval of 2 min. A single LV end-diastolic pressure value was determined by applying a horizontal cursor across the visually estimated end-diastolic pressure of five sequential LV pressure waveforms.

**Statistical Analysis**

Changes in salt intake, water intake, urine volume, urinary sodium, and PRA between the four groups were analyzed with the use of a two-way repeated-measures analysis of variance (ANOVA), followed by a post hoc Fisher's least-significant-difference test. Survival data were analyzed using a χ²-test of significance. A nonlinear regression program (SigmaPlot) was used to analyze the components of the individual sigmoid curve fits of the baroreflex data, and these values from the individual animals in each group were averaged to construct representative baroreflex curves, and these values from the individual animals in each group were averaged to construct representative baroreflex curves relating HR and RSNA changes to arterial pressure. Baseline values of RSNA, MAP, and HR, and the maximal gain and range values obtained from the baroreflex curve fits were analyzed using a one-way ANOVA, followed by a Student’s t-test, with differences considered significant at P < 0.05. Values are expressed as means ± SE.

**RESULTS**

**Echocardiographic Assessment of HF**

Baseline echocardiographic study demonstrated that the AV3V-x/MI rats (n = 14) and AV3V-s/MI rats (n = 14) had a comparable degree of LV injury (ischemic zone as % of LV wall: 49.6 ± 2.6 vs. 47.5 ± 2.5, AV3V-x vs. AV3V-s) [P = not significant (NS)], and comparable LVEF (0.37 ± 0.03 vs. 0.41 ± 0.05; P = NS), LVEDV (697 ± 34 vs. 699 ± 32 μl; P = NS), and LVEDV-to-mass ratios (LVEDV/M: 0.75 ± 0.1 vs. 0.71 ± 0.08; P = NS). The sham MI rats, AV3V-x/MI-s (n = 12) and AV3V-s/MI-s (n = 13), had no ischemic injury. These two groups also had similar values for LVEF (0.78 ± 0.03 vs. 0.80 ± 0.04, AV3V-x vs. AV3V-s; P = NS), LVEDV (341 ± 23 vs. 390 ± 26 μl; P = NS), and LVEDV/M ratio (0.45 ± 0.03 vs. 0.44 ± 0.05; P = NS).

y = yo + a/(1 + exp(−(x − xo)/b)), where x is the MAP; y is the change in (Δ) HR or ΔRSNA; a is the range of ΔHR or ΔRSNA; b is the slope coefficient; xo is MAP at the midpoint range of ΔRSNA or ΔHR; and yo is the minimum of ΔRSNA or ΔHR. In each rat, the raw data for MAP, HR, and RSNA were fit to this logistic function to generate parameters a, b, xo, and yo. The maximum gain of the baroreflex curve is defined as a/4b.

**Measurement of LV end-diastolic pressure.** After completion of the sympathetic recording, the animals were anesthetized with pentobarbital sodium (50 mg/kg ip), the right carotid artery was exposed, and a polyethylene-50 cannula attached to a pressure transducer was advanced through the carotid artery across the aortic valve into the LV chamber. Pressure was recorded continuously while the cannula was positioned at a site within the left ventricle at which LV pressure could be accurately recorded (i.e., the onset of the rapid rise in LV pressure after atrial contraction could be observed) and LV systolic pressure was not higher than aortic pressure on entering the LV (i.e., there was no evidence of ventricular outflow obstruction by the cannula). LV pressure was then recorded for an interval of 2 min. A single LV end-diastolic pressure value was determined by applying a horizontal cursor across the visually estimated end-diastolic pressure of five sequential LV pressure waveforms.

y = yo + a/(1 + exp(−(x − xo)/b)), where x is the MAP; y is the change in (Δ) HR or ΔRSNA; a is the range of ΔHR or ΔRSNA; b is the slope coefficient; xo is MAP at the midpoint range of ΔRSNA or ΔHR; and yo is the minimum of ΔRSNA or ΔHR. In each rat, the raw data for MAP, HR, and RSNA were fit to this logistic function to generate parameters a, b, xo, and yo. The maximum gain of the baroreflex curve is defined as a/4b.

**Measurement of LV end-diastolic pressure.** After completion of the sympathetic recording, the animals were anesthetized with pentobarbital sodium (50 mg/kg ip), the right carotid artery was exposed, and a polyethylene-50 cannula attached to a pressure transducer was advanced through the carotid artery across the aortic valve into the LV chamber. Pressure was recorded continuously while the cannula was positioned at a site within the left ventricle at which LV pressure could be accurately recorded (i.e., the onset of the rapid rise in LV pressure after atrial contraction could be observed) and LV systolic pressure was not higher than aortic pressure on entering the LV (i.e., there was no evidence of ventricular outflow obstruction by the cannula). LV pressure was then recorded for an interval of 2 min. A single LV end-diastolic pressure value was determined by applying a horizontal cursor across the visually estimated end-diastolic pressure of five sequential LV pressure waveforms.

**Statistical Analysis**

Changes in salt intake, water intake, urine volume, urinary sodium, and PRA between the four groups were analyzed with the use of a two-way repeated-measures analysis of variance (ANOVA), followed by a post hoc Fisher's least-significant-difference test. Survival data were analyzed using a χ²-test of significance. A nonlinear regression program (SigmaPlot) was used to analyze the components of the individual sigmoid curve fits of the baroreflex data, and these values from the individual animals in each group were averaged to construct representative baroreflex curves relating HR and RSNA changes to arterial pressure. Baseline values of RSNA, MAP, and HR, and the maximal gain and range values obtained from the baroreflex curve fits were analyzed using a one-way ANOVA, followed by a Student’s t-test, with differences considered significant at P < 0.05. Values are expressed as means ± SE.

**RESULTS**

**Echocardiographic Assessment of HF**

Baseline echocardiographic study demonstrated that the AV3V-x/MI rats (n = 14) and AV3V-s/MI rats (n = 14) had a comparable degree of LV injury (ischemic zone as % of LV wall: 49.6 ± 2.6 vs. 47.5 ± 2.5, AV3V-x vs. AV3V-s) [P = not significant (NS)], and comparable LVEF (0.37 ± 0.03 vs. 0.41 ± 0.05; P = NS), LVEDV (697 ± 34 vs. 699 ± 32 μl; P = NS), and LVEDV-to-mass ratios (LVEDV/M: 0.75 ± 0.1 vs. 0.71 ± 0.08; P = NS). The sham MI rats, AV3V-x/MI-s (n = 12) and AV3V-s/MI-s (n = 13), had no ischemic injury. These two groups also had similar values for LVEF (0.78 ± 0.03 vs. 0.80 ± 0.04, AV3V-x vs. AV3V-s; P = NS), LVEDV (341 ± 23 vs. 390 ± 26 μl; P = NS), and LVEDV/M ratio (0.45 ± 0.03 vs. 0.44 ± 0.05; P = NS).
The values for LVEF, LVEDV, and LVEDV/M ratio for the MI groups (AV3V-s and AV3V-x) were all significantly ($P < 0.05$) different from those of the MI-s groups (AV3V-s and AV3V-x). However, HR during echocardiography under sedation was not different across groups.

**Metabolic Cage Study**

**Volume regulation.** As shown in Fig. 1A, an AV3V lesion substantially reduced the increase in sodium appetite associated with MI. Within 1 wk after MI, rats with a sham AV3V lesion (AV3V-s/MI, $n = 8$) demonstrated a dramatic increase in consumption of 1.8% saline compared with those animals with no AV3V lesion and no MI (AV3V-s/MI-s, $n = 9$, the sham/sham group). In striking contrast, ingestion of the saline solution actually fell below that of the sham/sham group in the AV3V-x/MI rats ($n = 9$). The AV3V lesion had no effect on saline ingestion in MI-s rats ($n = 7$).

Water intake (Fig. 1B) did not change significantly in any of the four study groups. Although the volume of saline solution consumed must also be considered in assessing the total water intake, the volume of 1.8% saline consumed by the AV3V-s/MI group was <10% of the daily water intake.

As shown in Fig. 2A, the AV3V lesion prevented the renal sodium retention associated with MI. In the AV3V-s/MI rats, sodium excretion was markedly reduced ($P < 0.05$) compared with all other treatment groups. However, urinary sodium excretion in the AV3V-x/MI rats had not been different from that of the other three groups. MI rats with AV3V lesion had increased urine volume compared with MI-s rats with or without AV3V lesion. Values are means ± SE. *$P < 0.05$ vs. AV3V-s/MI-s; †$P < 0.05$, AV3V-x/MI vs. AV3V-s/MI.

**Sympathetic Nerve Recording Study**

**Baseline measurements.** Baseline recordings of RSNA and arterial pressure were obtained in conscious rats.
rats 4 h after recovery from a brief anesthesia to implant arterial and venous cannulas and renal nerve recording electrodes. Thus, although the animals received analgesics for pain, they were undoubtedly under some degree of postoperative stress.

Figure 4 shows fast time-base recordings from two rats with MI, one with (Fig. 4B) and one without (Fig. 4A) an AV3V lesion, illustrating both the nature of the sympathetic discharge in these rats and the manner in which the waveform average of RSNA was generated. In both tracings, the RSNA has a pulsatile quality related to the cardiac cycle, indicating persistent baroreflex modulation of RSNA. However, the magnitude of the sympathetic bursts is greater and less variable in the rat with the AV3V intact. In the AV3V-lesioned rat, the pulsatile quality is less dramatic, suggesting more prominent modulation by other factors (e.g., respiration).

The group data in Fig. 5 bear out a reduction in integrated voltage as well as in the frequency of bursting in the MI rats with AV3V-x compared with the MI rats with sham AV3V lesion. In fact, the AV3V lesion appears to have “normalized” these indexes of RSNA: that is, the results from MI rats with AV3V lesions are not different from the sham MI rats with sham AV3V lesion. Moreover, the AV3V lesion reduced (P < 0.05) the magnitude of the waveform average of RSNA/cardiac cycle in both the MI [AV3V-x vs. AV3V-s: 1.65 ± 0.07 (n = 5) vs. 2.09 ± 0.11 (n = 6) mV·s] and the MI-s [AV3V-x vs. AV3V-s: 1.41 ± 0.08 (n = 4) vs. 1.62 ± 0.10 (n = 5) mV·s] rats, demonstrating that the differences in RSNA across groups could not be attributed to the differences in HR. Again, the index of RSNA was “normalized” by the AV3V lesion. Interestingly, the AV3V lesion also lowered all
measures of sympathetic drive in the MI-s rats. Consistent with the reductions in RSNA, the AV3V lesion lowered the HR and arterial pressure in both the MI and MI-s groups (Fig. 5, A and B).

Baroreflex testing. Figures 6 and 7 show the logistic curve fits for baroreflex regulation of RSNA and HR, generated by averaging the values of the individual rats in each group; the mean values for maximum gain ($G_{max}$) and range of the baroreflex curves are shown in Fig. 8. The AV3V-s/MI rats ($n = 6$) had blunted baroreflex curves for HR and RSNA compared with the AV3V-s/MI-s rats ($n = 5$). In MI-s rats with AV3V-x ($n = 5$), $G_{max}$ and range of the baroreflex curves for RSNA and HR were reduced compared with AV3V-s/MI-s rats. In contrast, in MI rats with AV3V-x ($n = 6$), the $G_{max}$ and range of the baroreflex curves for RSNA and HR were improved compared with AV3V-s/MI rats.

Measurements of LV end-diastolic pressure. LV end-diastolic pressure (EDP) (mmHg) was higher ($P < 0.05$) in the MI groups (AV3V-x/MI: $16.49 \pm 1.75$, $n = 6$; AV3V-s/MI: $18.64 \pm 1.96$, $n = 6$) compared with the MI-s groups (AV3V-x/MI-s: $5.30 \pm 0.88$, $n = 5$; AV3V-s/MI-s: $4.39 \pm 1.01$, $n = 5$). The presence of an AV3V lesion had no significant effect on LVEDP within the MI-s or the MI groups.

Effect of AV3V Lesion on Survival Post-MI

Survival data were acquired on the animals in the metabolic cage study. Survival immediately after CL was comparable in the AV3V-lesioned rats (10/14, 71%) and the rats with sham AV3V lesions (12/16, 75%). However, the AV3V lesion had a pronounced effect on survival beyond 2 wk after CL. Among the 10 AV3V-lesioned rats surviving CL, only 2 (20%) were still alive 3 wk later, whereas 10 (83%) of 12 rats with sham AV3V lesions were alive. In the other groups, 8 (80%) of 10 AV3V-lesioned rats survived the sham CL surgery, and all of those (100%) were still alive 3 wk later; 7 of 7 (100%) rats with sham AV3V lesion survived the sham CL and all were alive at 3 wk. A $\chi^2$ test of significance indicated that the week 3 survival was reduced in the AV3V-x/MI rats ($P = 0.0013$).

Histology of the AV3V Lesion

The AV3V lesions were histologically verified as described previously. The lesions shared a common area of damage to the periventricular tissue between the anterior commissure and optic chiasm, including the ventral portion of organum vasculosum laminae terminalis (OVLT), the median preoptic nucleus, and anteroventral periventricular nuclei and the preoptic anterior hypothalamic periventricular nuclei. The medial preoptic and anterior hypothalamic nuclei sustained little damage beyond their medial borders with the periventricular nuclei.

DISCUSSION

The principal new finding of this study is that a lesion in the AV3V region of the forebrain dramatically alters the autonomic adjustments that seek to compensate for reduced heart function early after MI in the rat. After MI in rats with AV3V lesion, the expected increases in PRA and sympathetic drive (two important markers of neurohumoral excitation) did not occur. Physiological correlates included amelioration of the sodium consumption and renal sodium and water retention associated with
subjected to MI or MI-s. Mean RSNA in animals with AV3V-x or AV3V-s (Fig. 8) for baroreflex regulation of HR and B/H11006 and RSNA. Values are means lower HR and RSNA in MI than the MI-s rats. 

eliminating OVLT neurons and ing the OVLT and the median preoptic nucleus, thus 

The AV3V lesion damages ventral 

of the lesion on cardiovascular and autonomic regula- 

there is a substantial literature describing the effects 

mised after recovery from the acute adipsic phase, and 

compensatory autonomic mechanisms, survival was com- 

the circulatory adjustments to impaired myocardial func-

neural pathways within the AV3V region in mediating 

observations point to a critical role for neurons and/or 

of this region in regulating circulatory adjustments 

after MI. 

The AV3V lesion was chosen for this study because 

The AV3V lesion blocks the cardiovascular and au-

tonomic responses to acute administration of ANG II 

and the effects of acute dipsogenic stimuli that depend 

on conversion of ANG I to ANG II at forebrain CVOs: 

functional evidence that it interrupts descending path-

ways mediating the effects of the RAAS at forebrain 

CVOs. These same pathways presumably mediate the 

influences of other circulating peptides, e.g., endothe-

lin, the natriuretic peptides, that are released in the 

setting of diminished heart function (20) and may have 

differing cardiovascular and autonomic consequences. 

Moreover, it is likely that the AV3V lesion ablates 

neurons and fiber tracts that are tonically active, not 

dependent on humoral activation. For example, we 

recently demonstrated (29) that forebrain directed 

intracarotid injections of the angiotensin-converting en-

zyme inhibitor captopril reduces sympathetic drive in 

normal rats. 

In this perspective, perhaps the most intriguing find-

ing of the present study is the observation that the AV3V 

lesion did indeed affect the renin-angiotensin system but 

not in the anticipated manner. Instead of blocking the 
effects of an augmented peripheral renin-angiotensin 
system after MI, as anticipated, the AV3V lesion actually 

prevented the expected increase in PRA. Because renin 
release is the rate-limiting factor in the production of 
circulating ANG II, it may be inferred that the RAAS 
response to MI with reduced LV function was impaired in 
the AV3V-lesioned animal. 

The explanation for this finding is not immediately 

obvious. Renin renal release from the kidneys is deter-

mined by several factors (9), including renal perfusion, 

the intensity of RSNA and the levels of circulating 

ANG II. Our model of ischemia-induced HF involves a 
sizable area of myocardial injury. We had presumed 

that the resulting impairment of myocardial function 

would compromise renal blood flow, leading to renin 
release on that basis. The contrary finding in the 

AV3V-lesioned animals suggests that reduced renal 

blood flow is not the dominant factor determining renin 
release under conditions of MI and that other possibil-

ities need to be considered. The AV3V lesion also led to 

reduced sympathetic drive in the sham-MI rats, indi-

cating a tonic influence of neural elements within the 

AV3V region on sympathetic regulation. Rats with the 

AV3V lesion had less renal sympathetic nerve activity 
after MI than rats with the sham-AV3V lesion. It may 

be that the ambient level of renal sympathetic nerve 
discharge is the primary factor inducing renin release 
in our HF model. With the AV3V lesion, we may have 
interrupted a critical circuit linking the 
sympathetic nervous system and the renin-angiotensin 
system: e.g., sympathetic drive emanating from the 

forebrain drives renin release; ANG II in turn acts at 
forebrain to elicit further sympathetic drive and at the 
kidney to prevent further renin production and release. 

The improvement in baroreflex function induced by 

the AV3V lesion provides further insight into the role 
of this region in regulating circulatory adjustments 
after MI. Baroreflex blunting is a characteristic of both 
the post-MI state. Perhaps as a result of this failure of 
compensatory autonomic mechanisms, survival was com-

promised early after MI in the AV3V-lesioned rats. These 
observations point to a critical role for neurons and/or 
novel pathways within the AV3V region in mediating 
the circulatory adjustments to impaired myocardial func-

tion after MI.
region mediates baroreflex blunting provoked by ANG II infusion, an experimental condition simulating HF. Another study (30) in HF rats has shown normalization of sympathetic hyperactivity and baroreflex dysfunction after central angiotensin type 1 receptor blockade. Our MI rats with sham AV3V lesions had increased PRA, and thus increased circulating ANG II may well have contributed to their blunted baroreflexes. Baroreflex function was better (but still not normal) in the MI rats with an AV3V lesion. As previously discussed, the AV3V lesion in our preparation actually reduced renin release in response to MI and so operated by a somewhat different mechanism than by blocking effects of circulating ANG II. In any case, it would have eliminated any descending inhibitory influence the forebrain might have on baroreflex regulation of RSNA, ANG II mediated or not. Thus our results confirm a role for the AV3V region in the abnormal baroreflex response after MI, but suggest that it is not the only factor involved. In HF, both central (18) and peripheral (10) components of the baroreflex activity are said to be impaired.

Baroreflex function was also impaired in the MI-s rats with the AV3V lesion. This finding is at variance with previous results from normal rats (2). Whereas the explanation for this is uncertain, important factors may be differences in analytical technique (linear regression vs. logistic curve-fit analysis) and in timing of study: 2 to 3 wk post-AV3V lesion and 24 h after recovery from anesthesia in the former study; 8 wk after AV3V lesion, and 4 h after recovery from anesthesia in our study. In any case, this observation suggests that the AV3V region has a complex role with respect to baroreflex regulation. Considering the location and connectivity of the AV3V region, plasticity of neuronal function might occur depending on the nature of the neural and humoral signals impinging on it in particular pathophysiological states (e.g., HF and hypertension).

A particularly striking finding of the present study was that AV3V-x rats had a high mortality after MI compared with AV3V-s controls. Most of the AV3V-x/MI rats were dead at 3 wk, in contrast with the general pattern of survival in the other study groups. Thus, whereas interrupting forebrain mechanisms might theoretically confer long-term benefits by preventing the accumulation and retention of fluid that eventuates in HF, it is apparent that compensatory mechanisms mediated by the forebrain are essential to survival beyond the early post-MI period. Of course, any hypothalamic lesion has the potential to affect the multiple critical homeostatic mechanisms regulated by this region of the brain. On the basis of the known functions of this region of the brain, at least two possible contributing mechanisms might be suggested.

In HF, stroke volume is supported in part by increased filling of the dilated left ventricle. Thus one hypothesis might be that failure of the central mechanisms favoring sodium consumption and renal sodium and water conservation, as occurred after MI in the AV3V lesioned rats in the present study, compromises the ability of the animal to maintain adequate LV filling pressure. Insufficient LV filling associated with low ejection fraction and compromised sympathetic regulation of blood flow distribution to vital tissues may lead to hypotension, acidosis, accumulation of toxic metabolites, and ultimately tissue damage and death. Arguing against that hypothesis is the finding that the LV filling pressures were not low in AV3V-x/MI rats compared with the AV3V-s/MI rats studied 2 wk post-MI. Unfortunately, however, these LVEDP measurements were not obtained from the metabolic study group whose survival was assessed, but rather from the sympathetic nerve recording study group that was not maintained in metabolic cages. Thus a direct assessment of this parameter in animals in declining health was not available in this study.

An alternative hypothesis to explain the poor prognosis associated with superimposing coronary ligation on a background of the AV3V lesion is that other stress-related forebrain regulatory mechanisms activated by MI might be blocked by the AV3V lesion. For example, MI induces the release of proinflammatory cytokines (1), including tumor necrosis factor-α, which is increased in our experimental model of MI (14), and these agents signal the brain to augment the activity of the hypothalamic-pituitary-adrenal axis via increased production of corticotropin-releasing hormone in the paraventricular nucleus of the hypothalamus (19). Interference with this stress modulating process might well contribute to poor survival in the post-MI period. However, previous work has demonstrated that corticotropin-releasing hormone neurons are activated by cytokines (e.g., interleukin-1) over pathways not affected by interrupting the descending projections from CVOs of the lamina terminalis (11), and that corticosterone levels are actually increased and respond to stressors such as volume depletion in an exaggerated rather than a blunted manner after an AV3V lesion (5). Thus, although corticosterone levels were not measured in these studies, there is no precedent to suggest that insufficiency of the hypothalamic-pituitary-adrenal axis accounts for the poor survival of the AV3V-x/MI rats. Indeed, the normal survival of the AV3V-x/MI-s rats, and the time course (>2 wk) to death in the AV3V-x/MI rats would seem to argue strongly against a failure of the AV3V-lesioned rats to tolerate stress.

**Perspectives**

Our results support the general hypothesis that the forebrain is a critical component of the neural circuitry mediating the early adaptive responses to MI. The AV3V region is specifically implicated in the abnormalities of sympathetic drive and of volume regulation after MI with reduced LV function. Because the AV3V region can be affected by ascending inputs from the hindbrain regions that monitor cardiovascular afferent inputs and by descending inputs from forebrain CVOs that monitor humoral influences, the signal engaging this region of the brain after myocardial injury remains to be determined.
One clear message that emerges from this study, however, is that abrogating adaptive forebrain mechanisms has an adverse effect on survival after MI. Whereas these mechanisms may ultimately prove detrimental, eventuating in the excessive volume accumulation and augmented sympathetic drive that characterize end-stage HF, they appear to be essential to the early efforts of the body to compensate for reduced LV function. An important goal for future research will be to determine the best way to introduce a feedback signal indicating that optimal compensation for LV dysfunction has been achieved, thus interrupting the progression to overt HF.

The authors thank Kathy Zimmerman for diligent and expert assistance in the performance of the echocardiograms.

This study was supported by a Veterans’ Affairs Merit Review Award, National Heart, Lung, and Blood Institute (NHLBI) Grant HL-63915 (both to R. B. Felder), American Heart Association Grant-in-Aid 96-010430 (to R. M. Weiss), and NHLBI Cardiovascular Interdisciplinary Research Fellowship HL-07121 (to J. Francis).

REFERENCES


