Angiotensin II modulates catecholamine release into interstitial fluid of canine myocardium in vivo

DIANE M. FARRELL,1 CHIH-CHANG WEI,2 JOSÉ TALLAJ,2 JEFFREY L. ARDELL,3 J. ANDREW ARMOUR,4 GILBERT R. HAGEMAN,2 WAYNE E. BRADLEY,2 AND LOUIS J. DELL’ITALIA2

1Department of Physiology, University of Texas at San Antonio, San Antonio, Texas 78249; 2Division of Cardiovascular Disease, Department of Medicine, University Station, University of Alabama at Birmingham, Birmingham, Alabama 35294; 3Department of Pharmacology, East Tennessee State University, Johnson City, Tennessee 37614; and 4Department of Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada B3H 3J5

Received 29 November 1999; accepted in final form 30 March 2001

Farrell, Diane M., Chih-Chang Wei, José Tallaj, Jeffrey L. Ardell, J. Andrew Armour, Gilbert R. Hageman, Wayne E. Bradley, and Louis J. Dell’Italia. Angiotensin II modulates catecholamine release into interstitial fluid of canine myocardium in vivo. Am J Physiol Heart Circ Physiol 281: H813–H822, 2001.—This study tested the hypothesis that exogenous infusion of angiotensin II (ANG II) leads to the release of catecholamines (norepinephrine [NE] and epinephrine [EPI]) into the cardiac interstitial fluid (ISF) space of dogs with adrenals intact (AI) (n = 7) and with adrenals clamped (AC) (n = 5). LV ISF samples were collected at 3-min intervals during administration of ANG II (100 μM ANG II at 1 ml/min for 10 min) to right atrial neurons via their local arterial blood supply and during electrical stimulation of the stellate ganglia of open-chest anesthetized dogs. In AI dogs, ANG II caused ISF NE to increase fivefold (P < 0.05) without a significant increase in coronary sinus (CS) NE. Electrical stimulation (5 ms, 4 Hz, 8–14 V, and 10 min) of the stellate ganglia caused a similar increase in ISF NE (P < 0.05), accompanied by a sevenfold increase in CS NE (P < 0.05). ISF EPI increased greater than sixfold during ANG II infusion (P < 0.05) and during stellate stimulation. However, during ANG II infusions, aorta plasma EPI levels increased fourfold in AI dogs, whereas in AC dogs, CS NE and EPI levels were unaffected during ANG II infusions. Nevertheless, baseline ISF NE and EPI did not differ and increased to a similar extent during ANG II infusions in AI versus AC dogs. Thus exogenously administered ANG II increases the amount of NE liberated into the ISF independent of the adrenal contribution, the amount matching that induced by electrical stimulation of all cardiac sympathetic efferent neurons. In contrast, NE spillover into the CS occurred only during electrical stimulation of stellate ganglia. NE release and uptake mechanisms within the myocardium are differently affected, depending on how the final common pathway of the sympathetic nervous system is modified.

norepinephrine; epinephrine

IN ADDITION TO ITS WELL-KNOWN vasoconstrictor effect, angiotensin II (ANG II) acts as a growth factor for myocytes and fibroblasts (1, 29, 31). In the heart, activation of the ANG II type 1 (AT1) receptor mediates cardiac hypertrophy and fibrosis independent of hemodynamic and systemic neurohumoral levels (11). Thus the beneficial effects of AT1 antagonists and ANG-converting enzyme inhibitors in heart failure have been attributed in large part to inhibition of ANG II receptor function associated with cardiomyocytes and fibroblasts. AT1 receptors are associated not only with cardiomyocytes within the cardiac conduction system (28) but also with peripheral sympathetic neurons (8). In accord with functional data, AT1 receptor density in rabbit intrinsic cardiac ganglia is higher than in ventricular cardiomyocytes (6). ANG II is known to activate intracardiac neurons in situ (20) and to stimulate the release of norepinephrine (NE) from cardiac sympathetic nerves in vitro.

The anatomical relationship of AT1 receptors with cardiac neurons may account, in part, for the fact that ANG II-induced hypertension (12, 13) and myocyte necrosis and fibrosis (18, 19) are prevented by β-adrenergic receptor blockade. Furthermore, the myocyte necrosis and coronary vascular damage that occur within the first 3 days following elevation of circulating ANG II are prevented by AT1 receptor blockade. However, an ANG II-induced increase in serum NE does not occur until day 4 of exposure, and is prevented by AT1 receptor blockade (18, 19). These studies suggest that the deleterious effects of exogenously infused ANG II are mediated by enhanced local NE release via activation of AT1 receptors on cardiac adrenergic neurons.

ANG II induced positive chronotropic effects in adult guinea pig ventricular cardiomyocytes that were cultured with intrinsic cardiac or extracardiac sympathetic neurons but not in innervated cultured cardiomyocytes (20). These data support an ANG II-

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.
mediated functional relationship between cardiomyocytes and cardiac neurons. Furthermore, direct ANG II infusion into intrathoracic ganglia containing adrenergic neurons produced augmentation of heart rate as well as right ventricular and left ventricular (LV) inotropic that was blocked by pretreatment with either a β-adrenergic receptor blocker or an AT_1 receptor blocker (20). This study indicates that ANG II enhances cardiac contractile function by stimulating intrinsic cardiac adrenergic neurons to release NE into the cardiac interstitium.

Despite numerous studies indicating direct or indirect effects of ANG II on NE release, no study has demonstrated an ANG II-mediated increase in myocardial tissue NE in vivo. Accordingly, in the present study, we used the cardiac microdialysis technique to test the hypothesis that interstitial fluid (ISF) catecholamine levels can be modified by ANG II administered to intrinsic cardiac neurons, and that these levels are comparable to those achieved when intrathoracic sympathetic effector neurons are stimulated electrically in the dog.

**METHODS**

**Animal Preparation**

Twelve adult male mongrel dogs (25–30 kg) were screened to rule out *Ehrlichia canis platys* and *Dirofilaria immitis*. Animals were maintained on a deep plane of general anesthesia with the use of pentobarbital sodium and were mechanically ventilated (Harvard Apparatus; S. Natick, MA). Through a median sternotomy, the left and right stellate ganglia were isolated and decentralized. Stimulating electrodes were embedded into both of these ganglia. Both cervical left and right vagi were sectioned. The heart was then suspended in a pericardial cradle. An 8-Fr sheath (Cordis; Miami, FL) was inserted into the right carotid artery, and was positioned in the ascending aorta to sample aortic blood. A 5-Fr catheter transducer (Mikro-tip, Millar Instruments; Houston, TX) was inserted through this sheath into the LV for the measurement of LV pressure. Descending thoracic aortic pressure was monitored continuously with the use of a 3-Fr catheter transducer (Mikro-tip, Millar) inserted through a 7-Fr sheath implanted in the femoral artery. The femoral vein was cannulated for the administration of intravenous fluids. A 7-Fr catheter with multiple side holes was inserted into the coronary sinus through the left jugular vein to sample coronary sinus blood. A Doppler coronary flow probe (Transonic Systems; Ithaca, NY) was placed around the left anterior descending (LAD) coronary artery distal to the origin of the diagonal branch for coronary artery flow measurement. Four microdialysis probes were inserted into the LV myocardium in the region perfused by the LAD coronary artery at the base, middle, and apical regions of the anterior wall of the LV. This was done by the insertion of a curved 25-gauge needle through the myocardium and threading one end of the microdialysis probe inlet tubing through the needle. The needle was then withdrawn, and the probe was pulled through the tissue, thereby placing the dialysis membrane totally inside the musculature.

**Experimental Protocol in Animals With Adrenals Intact**

A flow diagram of the experimental protocol is shown in Fig. 1. After the preparatory surgery was completed, no interventions were performed during the following 2 h so that stress-induced tissue catecholamines could return to normal levels (2). During all periods, normal saline was infused through the microdialysis probes at a rate of 2.5 μl/min. In the first period (base 1), dialysate was collected for 10 min. In the second period (stimulate) the right and left stellate ganglia were stimulated (5 ms, 4 Hz, and 8–14 V) for 10 min. These stellate ganglia were decentralized from the central nervous system and stimulation voltage was set at two times the threshold to evoke a 10% increase in heart rate or LV contractile function. During the stimulation, dialysate was collected separately for the first 3 min (minutes 0–3), the second 3 min (minutes 3–6), and the last 4 min (minutes 6–10). During the 10 min after stimulation (post-stim), dialysate was collected separately for the first 5 min (minutes 0–5) and the second 5 min (minutes 5–10). This was followed by a 25-min washout, which was followed by a second 10-min baseline collection (base 2).

In the next period (ANG II), ANG II was infused into the right atrial ganglionated plexus neurons for 10 min (100 μM ANG II at 1 ml/min). This was done by securing a polyethylene-50 catheter in a side branch of the right coronary artery proximal to the origin of the arterial blood supply of right atrial neurons (20). During the infusions, dialysate was collected separately for the first 3 min (minute 0–3), the second 3 min (minute 3–6), and the last 4 min (minute 6–10). During the 10 min after infusion (post-ANG II) dialysate was collected for the first 5 min (minute 0–5) and the second 5 min (minute 5–10). This was followed by a 25-min washout, which was followed by a 10-min baseline collection (base 3). Samples were taken from aorta and coronary sinus blood 2 min before the end of base 1, 2 min before the end of stimulate, 2 min before the end of base 2, 2 min before the end of ANG II, and 2 min before the end of base 3. Mean arterial pressure (MAP), LV pressure, heart rate, and LAD coronary flow were measured throughout each procedure. LV first derivative of pressure development over time (dP/dt) was calculated during data analysis. Plasma and dialysate samples were assayed for ANG II, NE, and epinephrine (EPI).

**Experimental Protocol in Dogs With Adrenals Clamped**

Our initial data demonstrated that infusions of ANG II (100 μM of ANG II at 1 ml/min for 10 min) into the branch of the right coronary artery of a dog increased ISF ANG II and produced large increases in plasma ANG II (69,000 pg/ml). Accordingly, we studied five dogs with the adrenal glands removed from the circulation by cross clamping their vascular pedicles. The latter was done to negate the peripheral effects of ANG II on the release of catecholamines from the adrenal glands. Baseline ISF NE and EPI (base 1) as well as aorta and coronary sinus plasma NE and EPI were collected 2 h after the right and left adrenal glands were cross clamped, thereby isolating both adrenal glands from the vascular compartment. As in the protocol outlined in Fig. 1, ANG II was infused over 10 min into the right atrial ganglionated plexus neurons via a catheter inserted in a side branch of the right coronary artery. Dialysate was collected separately for minute 0–3, minute 3–6, and minute 6–10. During the 10 min after infusion (post-ANG II) dialysate was collected for minute 0–5 and minute 5–10. This was followed by a 25-min washout, which was followed by a 10-min baseline collection (base 2). This protocol was repeated during a 10-min infusion of ANG II into the ascending aorta (100 μM ANG II at 1 ml/min for 10 min). MAP, LV pressure, and heart rate were measured throughout each procedure. LV dP/dt was calculated during data analysis.
Cardiac Microdialysis

Each microdialysis probe (Clirans, Terumo; Tokyo, Japan) is a semipermeable membrane probe with a pore size of 35 Kd and an inner diameter of 200 μm, which is connected to methyl-deactivated silica capillary tubing with an outer diameter of 0.17 mm. Thus each microdialysis probe consists of a single dialysis fiber and two hollow silica tubes inserted, adjusted, and sealed within the dialysis fiber, such that the distance between the ends of the silica tubes is 4 cm. In each animal, four probes were implanted in the LV myocardium in the region perfused by the LAD coronary artery. After the microdialysis probes were inserted, the inflow capillary tube of each probe was connected via a larger deactivated silica tube to a gastight glass syringe filled with normal saline solution and perfused by a precision infusion syringe pump (BAS; W. Lafayette, IN) at 2.5 μl/min. The effluent, or dialysate, was collected from the outflow silica tube in 95% ethanol (μl) and was frozen (−80°C) until biochemical analysis.

Cardiac microdialysis is based on the principle that, as the dialysate solution passes through the microdialysis fiber, diffusion occurs between the fluid within the fiber and the ISF surrounding the fiber (10). The dialysate concentration is therefore an estimate of intramyocardial ISF concentration. At the flow rates used in vivo, however, it is unlikely that complete equilibration occurs between the normal saline solution within the fiber and the cardiac ISF in the vicinity of the fiber. To determine perfusion speed, we performed in vitro experiments to determine the relationship between perfusion speed and relative recovery. A dialysis probe was bathed in 37°C saline solution with a constant NE concentration of 10 ng/ml, and dialysate samples were collected at various perfusion speeds (Fig. 2). Dialysate NE concentration was measured by high-performance liquid chromatography (HPLC). At 2.5 μl/min, the rate that was used for the present in vivo experiments, the percent recovery was 18.8 ± 1.7%. Therefore, 18.8% was used in the final calculation of ISF values, and, thus represents an estimate of ISF levels because diffusional exchange may differ between a beaker and the beating heart. We have also assessed (10) in vivo stability of our microdialysis probes by determining the concentration of the stable compound acetaminophen in the probe effluent.

Fig. 1. Diagram of experimental protocol. Two hours were allowed to pass between preparatory surgery and experimental measurements. During all periods, normal saline was infused through the microdialysis probes. Base 1, dialysate collected during no intervention period; Stimulate, dialysate collected at minutes 0–3, 3–6, and 6–10 during stimulation of both stellate ganglia; Post-Stim, dialysate collected at minutes 0–5 and 5–10 immediately after stimulation; Base 2, dialysate collected during no intervention; angiotensin II (ANG II), dialysate collected at minutes 0–3, 3–6, and 6–10 during infusion of ANG II into a branch of the right coronary artery; Post-ANG II, dialysate collected at minutes 0–5 and 5–10 immediately after ANG II infusion; and Base 3, dialysate collected during no intervention. Aortic and coronary sinus blood samples were collected at the end of each period where noted.
Concentrations of acetaminophen were calculated by using HPLC analysis of the dialysis probe infusate and the dialysis probe effluent. The initial concentration in the infusate was considered to be 100%. We found that 83% of acetaminophen was detected in the effluent, indicating that 17% diffused into the ISF because the compound is not degraded under these conditions.

Preliminary data in our laboratory have demonstrated that ISF NE levels increased to a greater extent at the base than at the apex (P < 0.05); whereas ANG II-induced increases in NE tended to be higher in the base of the heart in dogs with adrenals intact. Accordingly, all ISF NE and EPI samples were drawn from the base of the heart from a single dialysis probe from each dog.

Biochemical Analyses

Angiotensin peptide levels. ISF and plasma ANG I and ANG II concentrations were determined by a method described from our laboratory that combines solid-phase extraction, HPLC, and radioimmunoassay (10). AG50WX4 (200–400 mesh) cation exchange resin was used in a solid-phase extraction procedure for sample purification. The recovery from the solid-phase extraction procedure has been determined previously (10) in our laboratory by using both labeled and unlabeled ANG peptides. With the use of 125I-labeled ANG I (1.4 × 10^7 cpm) and 125I-labeled ANG II (9 × 10^6 cpm), recoveries were 91 ± 9% and 93 ± 1% (n = 6), respectively. Separation was performed by reversed-phase HPLC on a phenyl silica gel column with an eluent consisting of 20% acetonitrile in 0.1 M ammonium phosphate buffer, pH 4.9. Each HPLC fraction was 300 μl. Aliquots (100 μl) of each relevant fraction of column effluent were subjected to radioimmunoassay immediately on collection. Elution of standard ANG peptides under isocratic conditions revealed clear resolution of ANG I, II, and III, and ANG(1–7) and ANG(3–8) peptides. Radioimmunoassay of relevant peaks revealed detectable levels of ANG I and II in all of the plasma and ISF samples examined. Antibodies to ANG I and II were raised in our laboratory for New Zealand White rabbits immunized against peptides conjugated to poly-L-lysine, as previously described (10). Cross-reactivity of anti-ANG I antiserum with ANG II and of anti-ANG II antiserum with ANG I was <0.5%. The sensitivity of the radioimmunoassay for ANG I was 4 pg/ml and for ANG II was 2 pg/ml.

Catecholamine levels. ISF and plasma NE and EPI concentrations were determined with the Biotrak catecholamines 3H-labeled radioenzymatic assay (Amersham Pharmacia Biotech). NE and EPI were simultaneously converted to their corresponding meta 3H-labeled methoxy derivative by the catalytic action of a partially purified preparation of catechol-O-methyltransferase in the presence of S-adenosyl-L-[3H]-methionine. After O-methylation, the catecholamine derivatives were extracted and separated by thin-layer chromatography. Each labeled derivative was converted by periodate oxidation to [3H]vanillin, extracted and scintillation counted.

Statistical Analysis

All data are presented as means ± SE. One-way repeated measures analysis of variance with post hoc comparison was used to compare hemodynamics and EPI and NE concentrations throughout the stages of the protocol. P < 0.05 was considered significant.

RESULTS

ISF NE Levels in Dogs With Adrenals Intact

Stellate ganglion stimulation. After 3 min of stellate ganglia stimulation, ISF NE increased from 457 ± 65 pg/ml to 1,606 ± 122 pg/ml (P < 0.05) and increased even further during the next 3 min (2,173 ± 318 pg/ml; P < 0.05 vs. base). NE concentration in ISF collected during the last 4 min of stimulation fell to 1,470 ± 442 pg/ml (P < 0.05 vs. base) (Fig. 3A). This index returned to baseline values after cessation of stimulation (460 ± 61 and 450 ± 19 pg/ml, respectively).

ANG II infusion. ISF NE levels increased from 441 ± 88 to 725 ± 159 pg/ml in the first 3 min of local coronary administration. NE increased further (2,178 ± 922 pg/ml, P < 0.05) during the second 3-min period of ANG II infusion. NE levels were 1,072 ± 443 pg/ml during the last 4 min of infusion, returning to baseline values by 5 min after cessation of the infusion (5 min, 352 ± 76; 10 min, 302 ± 76 pg/ml).

ISF EPI Levels in Dogs With Adrenals Intact

Stellate ganglion stimulation. During stellate stimulation, ISF EPI levels increased from 215 ± 58 to 646 ± 266 pg/ml at 3 min, increasing to 1,780 ± 951 pg/ml by 6 min of stimulation. ISF EPI fell back to baseline values (246 ± 47 pg/ml) during the last 4 min of stimulation. EPI remained at baseline values 5 and 10 min after cessation of stimulation (200 ± 88 and 181 ± 43) (see Fig. 3B).

ANG II infusion. During local ANG II infusion, ISF EPI increased from 280 ± 53 to 1,063 ± 106 pg/ml. While ANG II infusion continued, EPI increased significantly (1,773 ± 482 pg/ml, P < 0.05) in the second 3 min and remained elevated during the last 4 min of infusion (1,382 ± 525 pg/ml, P < 0.05 vs. baseline). These values returned to baseline levels 5 and 10 min...
Aortic and Coronary Sinus Plasma NE and EPI Levels in Dogs With Adrenals Intact

Stellate ganglion stimulation. Stellate ganglia stimulation led to an increase in coronary sinus NE (145 ± 23 to 1,414 ± 438 pg/ml; P < 0.01), which was greater than that which occurred in aortic plasma (from 205 ± 34 to 551 ± 58 pg/ml, P < 0.01) (Fig. 6A). These data demonstrate a net release of NE by the heart. In contrast, aortic and coronary sinus EPI levels were not significantly different from those at baseline levels (Fig. 6B).

ANG II infusion. Both aortic and coronary sinus plasma EPI levels increased significantly during ANG II infusion. Aortic EPI increased fourfold from 392 ± 154 to 1,876 ± 431 pg/ml (P < 0.01), whereas coronary sinus EPI increased from 301 ± 164 to 938 ± 251 (P < 0.05). Aortic EPI levels were greater than coronary sinus levels during ANG II infusion suggesting a net uptake of EPI by the heart. In contrast, aortic and coronary sinus NE levels were not significantly different from baseline levels.

after cessation of ANG II infusion (5 min, 396 ± 62; 10 min, 213 ± 37).

Hemodynamics in Dogs With Adrenals Intact

Stellate ganglion stimulation. Stellate ganglia stimulation increased heart rate, LAD flow, peak LV pressure, and mean LV dP/dt. Heart rate and LAD coronary artery flow increased throughout the stimulation period with variable significance (Fig. 4). MAP increased slightly during stellate stimulation but only achieved significance at 4 min. In contrast, LV dP/dt was significantly increased at all times except minute 10 (Fig. 5A).

ANG II infusion. ANG II infusion resulted in early increases in LAD flow, whereas heart rate was unchanged (Fig. 4). MAP was increased throughout the 10-min infusion, whereas dP/dt increased only at minute 4 (Fig. 5B).
ISF NE Levels in Dogs With Adrenals Clamped During ANG II Infusion

**Intracoronary ANG II infusion.** ISF NE levels increased from 613 ± 61 to 1,977 ± 418 pg/ml (P < 0.05) and remained elevated at 6 and 10 min of infusion (2,045 ± 602 and 1,346 ± 232 pg/ml, P < 0.05 vs. baseline). ISF NE returned to baseline levels at the end of ANG II infusion (Fig. 7A).

**Intra-arterial ANG II infusion.** ISF NE levels increased significantly from 370 ± 138 at baseline to 2,344 ± 515 (P < 0.05) and 1,597 ± 568 (P < 0.05) after 6 and 10 min of infusion, respectively.

Hemodynamics in Dogs With Adrenals Clamped During ANG II Infusion

**Intracoronary ANG II infusion.** ANG II infusion increased MAP and dP/dt during the first 4 min of the infusion (P < 0.05). The MAP and dP/dt started decreasing by 6 min of infusion and subsequently decreased to baseline levels during the last 2 min despite continued ANG II infusion (Fig. 8A).

**Intra-arterial ANG II infusion.** ANG II infusion resulted in an increased MAP and dP/dt after 3 min (P < 0.05) but decreased at 6 min and returned to baseline levels at 8 and 10 min of ANG II infusion (Fig. 8B).

Aortic and Coronary Sinus NE and EPI Levels in Dogs With Adrenals Clamped During ANG II Infusions

Baseline plasma EPI and NE levels did not differ in dogs with intact adrenals or cross-clamped adrenals. However, in dogs with cross-clamped adrenals, ANG II...
infusions into the right coronary artery or ascending aorta did not change plasma EPI levels (Fig. 9).

**DISCUSSION**

To our knowledge, the current investigation represents the first to study the relationship between catecholamine levels in the myocardial ISF and the coronary sinus blood during electrical- versus ANG II-induced activation of cardiac sympathetic efferent neurons in the normal dog heart in vivo. The main finding of the present study is that exogenous administration of ANG II to a population of intrinsic cardiac neurons leads to an increase release of NE and EPI into the cardiac ISF space, independent of peripheral systemic effects mediated by adrenal glands. The level of NE in the ISF during exogenous ANG II infusion was equivalent to ISF NE levels achieved during high-level electrical stimulation of the stellate ganglia (i.e., all sympathetic efferent neurons). However, stellate stimulation caused a significant amount of NE to spillover into the coronary sinus, whereas ANG II did not. These results suggest that NE release and uptake mechanisms within the myocardium can be differentially affected, depending on how the final common pathway of the sympathetic efferent nervous system is modified.

Electrical stimulation of cardiac sympathetic efferent neurons has been shown to cause a spillover of NE into coronary sinus blood (26, 27) or an increase of NE in the cardiac ISF (2, 21) in vivo. Furthermore, Kawada et al. (21) showed that ISF NE levels correlate with measures of ventricular contractility elicited during stellate ganglia stimulation in the cat in vivo. It is of interest that the increases in coronary sinus NE levels and the positive inotropic response achieved by stellate ganglion stimulation are markedly decreased in dogs with chronic pacing-induced heart failure compared with normals (7). Similarly, Armstrong et al. (3) reported that intracardiac NE content decreased by 50% in dogs with rapid pacing-induced heart failure. Taken together, these studies support the idea that cardiac NE stores and NE release mechanisms play an important part in inotropic support of the normal and failing heart.

Fig. 7. Interstitial fluid NE (A) and EPI (B) levels during a 10-min infusion of ANG II into a branch of the right coronary artery (IC Inf., left) and during a 10-min infusion of ANG II into the ascending aorta (IA Inf., right) of dogs with adrenals clamped. *P < 0.05 vs. baseline value of IC Inf. and IA Inf.

Fig. 8. Left ventricular dP/dt and mean arterial pressure (MAP) during a 10-min infusion of ANG II into a branch of the right coronary artery (A) and during a 10-min infusion of ANG II into the ascending aorta (B) in dogs with adrenals clamped. *P < 0.05 vs. minute 0.
We found that cardiac stores of EPI can be released into the ISF on activation of cardiac adrenergic efferent neurons either by electrically stimulating stellate ganglia or by administering ANG II to the intrathoracic cardiac nervous system. The EPI so released into the ISF can directly activate cardiac myocyte β1-adrenergic receptors, thereby augmenting cardiac chronotropism and inotropism. Neuroally released EPI can likewise activate β1- and β2-receptors on intrinsic cardiac adrenergic efferent neurons to enhance their release of NE into the interstitium (27). The importance of the modulator effects of EPI on intracardiac neuronal NE release was recently demonstrated in patients with chest pain syndromes and normal ventricular function. In that study (24), intracoronary administration of a β2-selective agonist was associated with increased cardiac NE spillover that was reduced by a selective β1-receptor antagonist and prevented by a nonselective β-receptor antagonist. Taken together, these data indicate that ANG II infusion enhances catecholamine release via activating AT1 receptors associated with cardiac adrenergic efferent neurons, both NE and EPI contributing to the subsequent augmentation in regional cardiac function. Furthermore, alterations in ventricular interstitial catecholamine content may affect the intrinsic cardiac nervous system directly to further enhancing neuronal release of catecholamines into the interstitial space in a synergistic manner.

The level of NE liberated in the ISF during exogenous administration of ANG II to a population of intrinsic cardiac neurons was equivalent to that achieved during electrical stimulation of all intrathoracic sympathetic efferent neurons. Studies (5, 14, 23) of isolated rat, pig, and mouse atria have demonstrated that ANG II-mediated increases of NE can be blocked by AT1 receptor antagonists. An in vivo dog study demonstrated that infusion of ANG II into the left coronary artery enhanced coronary sinus output of NE after sympathetic efferent neuronal activation. However, ANG II infusion alone had no effect on the basal output of NE from the heart (22). In human subjects, IV infusion of pressor doses of ANG II does not result in increased NE spillover in plasma. In the present study, we found that infusion of pharmacological doses of ANG II into the arterial blood supply of right atrial neurons caused a threefold increase in ISF NE without spillover of NE into the coronary sinus. The increase in ISF NE was similar to that induced in dogs with adrenals clamped, suggesting that local release and rapid reuptake mechanisms of NE can be activated by ANG II-sensitive intrinsic cardiac neurons, independent of ANG II-stimulation of the adrenal glands or other extracardiac neurons.

Such an effect of locally produced ANG II on ISF NE and EPI, as in the pathophysiology of cardiac hypertrophy and heart failure, can have a deleterious effect on LV structure and function. Senzaki et al. (30) showed that ANG II, when infused for 4 days, followed by 48 h of pacing (during continued ANG II infusion), markedly enhances LV dysfunction. This effect can be prevented by pretreatment with a high dose of a selec-
tive β-receptor blocker. Henegar et al. (18, 19) showed that cardiac damage associated with increased ANG II could be minimized by β1-adrenergic blockade or sympathectomy. ANG II induces an increase in serum NE but only after 4 days of exposure. These data suggest that the deleterious effects of exogenously administered ANG II are mediated by enhanced NE release that the deleterious effects of exogenously administered ANG II are mediated by enhanced NE release secondary to activation of AT1 receptors on cardiac adrenergic neurons. Furthermore, continuous ANG II infusion caused a progressive decrease in β1-adrenergic receptor density in these rat hearts. Asano et al. (4) found a positive correlation between AT1- and β1-receptor densities in failing and nonfailing human hearts, suggesting that downregulation of these two receptor systems is related pathophysiologically, both being modulated by ANG II and NE.

Increases in the activities of both the renin-angiotensin and sympathetic nervous systems are pathogenic of congestive heart failure. The initial increases in cardiac rate, contractility, and total peripheral resistance are compensatory, increasing blood pressure and venous return. However, long-term activation of the renin-angiotensin system and the sympathetic eff erent nervous system has adverse consequences that ultimately exacerbate the deleterious effects of heart failure. The results of the current investigation provide further evidence that ANG II can modulate cardiac ISF levels of NE and EPI, independent of its direct cardiomyocyte or systemic vascular effects. Over time, this can result in a destructive positive feedback cycle that can have deleterious effects on cardiac structure and function. Whether pathophysiological states achieve concentrations of ANG II in the cardiac interstitium that can modulate ISF catecholamines is the goal of future investigations utilizing animal models of heart failure.

The authors thank Joan Durand for performing the radioimmunoassays and Pamela Gibson for expert secretarial skills in preparing this manuscript. This study was supported by a grant from the Department of Veteran Affairs Office of Research and Development, Medical Service (to L. J. Dell’Italia), National Heart, Lung, and Blood Institute Grants RO1-HL-54816 (to L. J. Dell’Italia) and HL-58140 (to J. L. Ar dell), and by an American Heart Association Southeast fellowship (to C-C. Wei).

REFERENCES


24. Newton GE and Parker JD. Acute effects of β1-selective and nonselective β-adrenergic receptor blockade on cardiac sympa-

AJP-Heart Circ Physiol • VOL 281 • AUGUST 2001 • www.ajpheart.org


