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Exogenous Angiotensin II Does Not Facilitate Norepinephrine Release in the Heart

Thomas W. Lameris, Sandra de Zeeuw, Dirk J. Duncker, Gooitzen Alberts, Frans Boomsma, Pieter D. Verdouw, Anton H. van den Meiracker

Abstract—Studies on the effect of angiotensin II on norepinephrine release from sympathetic nerve terminals through stimulation of presynaptic angiotensin II type 1 receptors are equivocal. Furthermore, evidence that angiotensin II activates the cardiac sympathetic nervous system in vivo is scarce or indirect. In the intact porcine heart, we investigated whether angiotensin II increases norepinephrine concentrations in the myocardial interstitial fluid (NE_{MIF}) under basal conditions and during sympathetic activation and whether it enhances exocytotic and nonexocytotic ischemia-induced norepinephrine release. In 27 anesthetized pigs, NE_{MIF} was measured in the left ventricular myocardium using the microdialysis technique. Local infusion of angiotensin II into the left anterior descending coronary artery (LAD) at consecutive rates of 0.05, 0.5, and 5 ng/kg per minute did not affect NE_{MIF} , LAD flow, left ventricular dP/dt_{max} , and arterial pressure despite large increments in coronary arterial and venous angiotensin II concentrations. In the presence of neuronal reuptake inhibition and α -adrenergic receptor blockade, left stellate ganglion stimulation increased NE_{MIF} from 2.7 ± 0.3 to 7.3 ± 1.2 before, and from 2.3 ± 0.4 to 6.9 ± 1.3 nmol/L during, infusion of 0.5 ng/kg per minute angiotensin II. Sixty minutes of 70% LAD flow reduction caused a progressive increase in NE_{MIF} from 0.9 ± 0.1 to 16 ± 6 nmol/L, which was not enhanced by concomitant infusion of 0.5 ng/kg per minute angiotensin II. In conclusion, we did not observe any facilitation of cardiac norepinephrine release by angiotensin II under basal conditions and during either physiological (ganglion stimulation) or pathophysiological (acute ischemia) sympathetic activation. Hence, angiotensin II is not a local mediator of cardiac sympathetic activity in the in vivo porcine heart. (*Hypertension*. 2002;40:491-497.)

Key Words: norepinephrine ■ angiotensin II ■ renin-angiotensin system ■ sympathetic nervous system

Activation of the sympathetic nervous system simultaneously leads to activation of the renin-angiotensin system via stimulation of β -adrenergic receptors within the kidney, resulting in an increased renin release. There is also evidence, albeit conflicting, that the sympathetic nervous system is activated by the renin-angiotensin system.¹⁻¹³ This activation supposedly occurs through stimulation of angiotensin II receptors within the central nervous system and/or stimulation of presynaptic angiotensin II receptors located at sympathetic nerve terminals. When investigating the sympathetic nervous system and its interaction with the renin-angiotensin system, the heart is of particular interest. First of all, the mammalian heart has a dense sympathetic innervation. Second, all components of the renin-angiotensin system are present in the heart, and most angiotensin II in the heart is formed from locally synthesized angiotensin I.¹⁴ Third, in conditions like hypertension, ischemia, and especially heart failure, the renin-angiotensin system and sympathetic nervous system are both activated, and this activation likely contributes to the deterioration of cardiac function.¹⁵⁻¹⁷

Evidence that angiotensin II activates the cardiac sympathetic nervous system in vivo is scarce⁴ or indirect.^{7,18} In a recent study, Teisman et al⁴ have shown with the use of the microdialysis technique that “pharmacological” (10^{-6} mol/L) concentrations of locally applied angiotensin II were associated with an increase in norepinephrine concentrations in the myocardial interstitial fluid (NE_{MIF}) of the in vivo rat heart. In the present study, we determined whether “physiological” (10^{-10} mol/L) to “pathophysiological” (10^{-8} mol/L) concentrations of angiotensin II modulate NE_{MIF} in the intact porcine heart. The pig is especially suitable as a model for studying the cardiac sympathetic nervous system because, unlike the rat, the prevailing parasympathetic control of cardiac function is very similar to that in man, which allows for a more reliable extrapolation of the experimental results to the reality of human patients.

To exclude a masking effect of neuronal norepinephrine reuptake and negative feedback through presynaptic α -adrenergic receptor stimulation on modulation of NE_{MIF} by angiotensin II, we co-perfused some probes with the U1-

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inhibitor desipramine and the α -adrenergic receptor-blocker phentolamine without provoking systemic hemodynamic effects that also may modulate norepinephrine release.^{19–21}

In those studies that demonstrated interaction between angiotensin II and the sympathetic nervous system, most evidence points toward direct facilitation mediated by presynaptic angiotensin II type 1 (AT₁) receptors resulting in either a classic calcium-dependent augmentation of exocytotic norepinephrine release^{1–3} or in enhanced nonexocytotic release via activation of the Na⁺/H⁺ exchanger.^{22,23} Therefore, we not only investigated the modulation of NE_{MIF} by angiotensin II under basal conditions but also during enhanced exocytotic norepinephrine release evoked by stimulation of the left stellate ganglion. In addition, we monitored norepinephrine release in MIF during reduction of left anterior descending coronary artery (LAD) flow, resulting in both exocytotic and nonexocytotic norepinephrine release,²⁰ while still allowing for intracoronary infusion of angiotensin II.

Methods

Animal Procedures

All experiments were performed in accordance with “Guiding Principles for Research Involving Animals and Human Beings” as approved by the Council of the American Physiological Society and under the regulations of the Animal Care Committee of the Erasmus University Rotterdam.

Crossbred Landrace×Yorkshire pigs of either sex (30 to 35 kg, n=27) were used. Treatment, surgical procedure, and positioning of catheters and flow probes have been described previously.^{19,20} In animals subjected to LAD flow reduction, a fluid-filled balloon occluder (In Vivo Metric) was placed around the LAD distal to the Doppler flow probe and attached to a bidirectional roller pump (Ismatec). The voltage output from the Doppler equipment was directed through a custom-built electrical circuit, which steered the roller pump to maintain LAD flow at 30% of baseline values. In animals subjected to sympathetic stimulation, the left stellate ganglion was dissected, and an electrode was inserted into the ganglion, as described by Gootman et al.²⁴ and connected to a nerve stimulator (Grass S9; pulses of 12 V, 10 Hz, and 5 ms).

Microdialysis probes were implanted in the left ventricular (LV) myocardium: 1 in the region perfused by the left circumflex coronary artery (LCx) to determine NE_{MIF,LCx} and 3 in the area perfused by the LAD to determine NE_{MIF,LAD}. To achieve local U1-inhibition, one of the LAD probes was co-perfused with desipramine (100 μ mol/L, Sigma),²¹ while another LAD probe was co-perfused with desipramine and phentolamine (100 μ mol/L, Department of Pharmacy, University Hospital Dijkzigt, Rotterdam) to block presynaptic α -adrenergic receptor-mediated inhibition of norepinephrine release. The microdialysis technique, probe characteristics, handling of the microdialysis and plasma samples for the measurement of norepinephrine concentrations, and probe recovery have been described previously.^{19,25} Plasma samples for determination of angiotensin II concentrations (AngII) were rapidly drawn into chilled plastic syringes containing an “inhibitor mix.”¹⁴

Experimental Protocol

After a 120-minute stabilization period, baseline measurements were obtained over a 30-minute period. Probes were perfused with Ringer’s solution (Baxter) at a flow of 2 μ L/min, and dialysate was collected at 10-minute intervals, during which period blood was collected from the aorta and the interventricular coronary vein.^{19,20} In group I (n=7), the effects of angiotensin II on basal sympathetic norepinephrine release were investigated by infusing angiotensin II (Department of Pharmacy) into the LAD at consecutive infusion rates of 0.05, 0.5, and 5 ng/kg per minute for 20 minutes each. In group II (n=7), we assessed the effects of angiotensin II on enhanced

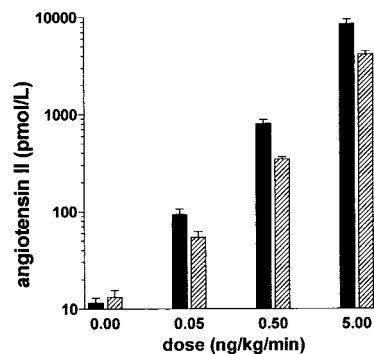


Figure 1. Intracoronary angiotensin II infusions and plasma angiotensin II concentrations in the interventricular coronary vein (hatched bars) and left anterior descending coronary artery (solid bars). Data are mean \pm SEM, n=7.

exocytotic norepinephrine release by stimulating the left stellate ganglion before and during infusion of angiotensin II. To investigate the effect of angiotensin II on nonexocytotic norepinephrine release, the LAD flow was reduced by 70% for 60 minutes without (group III, n=6) and during simultaneous infusion of angiotensin II into the LAD (group IV, n=7). After 120 minutes of reperfusion, the LAD perfusion area (area at risk) and infarct size were determined.²⁰

Analytical Procedures

Norepinephrine concentrations in plasma and microdialysis samples were determined by high performance liquid chromatography with fluorometric detection.²⁵ Plasma angiotensin II concentrations were determined with high performance liquid chromatography after Sep-Pak extraction and radioimmunoassay.¹⁴

Data Analysis and Statistics

Dialysate norepinephrine concentrations were corrected for probe recovery to yield norepinephrine concentrations in MIF.^{19,20} Lower limits of detection for norepinephrine in dialysate and plasma were 0.2 and 0.02 nmol/L, respectively.²⁵ Baseline values were determined by averaging the 3 measurements over the 30-minute period before intervention.^{19,20} Angiotensin II plasma concentrations in the LAD were calculated from angiotensin II infusion rate, coronary plasma flow (LAD_{flow}×[1-hematocrit]), and arterial angiotensin II concentrations. Results are expressed as mean \pm SEM. For statistical analysis two-way analysis of variance, one-way analysis of variance for repeated measures with Dunnett’s multiple comparison test as post hoc test, and Student *t* test were used as appropriate.

Results

Intracoronary Angiotensin II Infusion and Basal Norepinephrine Concentrations (Group I)

During infusion of angiotensin II, angiotensin II concentrations in the LAD and the coronary vein rose from 12 \pm 1 and 13 \pm 2 pmol/L at baseline up to 8485 \pm 1082 and 4150 \pm 329 pmol/L during infusion of 5 ng/kg per minute, respectively (Figure 1), while aortic angiotensin II concentrations increased from 12 \pm 1 to 80 \pm 9 pmol/L. Despite these large increments in angiotensin II concentrations, there were no significant changes in global hemodynamics (Table 1), NE_{MIF}, or arterial and coronary venous norepinephrine concentrations (Table 2 and Figure 2). However, LAD flow tended to decrease, which necessitated increased myocardial O₂ extraction, resulting in a decrease in coronary venous O₂ saturation (Table 1).

TABLE 1. Cardiovascular Function During Intracoronary Infusion of Angiotensin II

	Baseline	Angiotensin II, ng/kg per minute		
		0.05	0.5	5
Mean arterial pressure, mm Hg	83±2	80±5	83±3	85±3
Cardiac output, L/min	2.4±0.1	2.2±0.1	2.4±0.1	2.3±0.1
Heart rate, bpm	134±5	135±6	135±6	134±6
LV dP/dt _{max} , mm Hg/sec	1552±76	1472±111	1547±87	1551±87
LV end diastolic pressure, mm Hg	12±1	11±1	11±1	12±1
LAD flow, mL/min	32±5	31±4	30±3	28±3
O ₂ Saturation, %	28±4	25±2	21±2*	21±1*

Data are mean±SEM, n=7.

*P<0.05 versus baseline.

Intracoronary Angiotensin II Infusion and Norepinephrine Release During Sympathetic Activation (Group II)

Stimulation of the left stellate ganglion caused marked increases in blood pressure (19%), LAD flow (25%), and, in particular, LV dP/dt_{max} (190%, Table 3 and Figure 3) and caused a rise in NE_{MIF}, particularly in the presence of U1- and α-adrenergic receptor blockade where NE_{MIF,LAD} increased from 2.7±0.4 to 7.3±1.2 nmol/L (Table 2 and Figure 3). Concomitant intracoronary infusion of angiotensin II did not affect hemodynamic responses to stimulation, nor did it

modify the stimulation-induced increase in NE_{MIF,LAD} (from 2.3±0.3 to 6.9±1.3 nmol/L).

Intracoronary Angiotensin II Infusion and Norepinephrine Release During Ischemia (Groups III and IV)

Cardiovascular Function

The 70% LAD flow reduction resulted in 10% reductions of mean arterial pressure and cardiac output, whereas LV end-diastolic pressure slightly increased (Table 4). Following reperfusion, mean arterial pressure and cardiac output re-

TABLE 2. Effect of Intracoronary Angiotensin II Infusion on Circulatory and Interstitial Norepinephrine Concentrations

	Arterial Plasma (nmol/L)	Coronary Vein (nmol/L)	MIF LAD			
			Control (nmol/L)	DMI (nmol/L)	DMI+PHA (nmol/L)	MIF LCx (nmol/L)
Group I (n=7)						
Baseline	0.4±0.2	0.4±0.1	0.6±0.1	1.4±0.2†‡§	2.3±0.4†‡§	0.5±0.2
AngII 0.05	0.5±0.1	0.3±0.1	0.6±0.1	1.3±0.2†‡§	2.1±0.2†‡§	0.4±0.1
AngII 0.5	0.4±0.2	0.4±0.1	0.5±0.1	1.2±0.2†‡§	2.1±0.4†‡§	0.6±0.1
AngII 5	0.3±0.1	0.3±0.1	0.6±0.2	1.3±0.2†‡§	2.2±0.5†‡§	0.6±0.1
Group II (n=7)						
Baseline	0.1±0.0	0.2±0.1	0.5±0.1†‡	1.7±0.4†‡§	2.7±0.3†‡§	0.6±0.1†‡
LSG stimulation	1.0±0.1*	1.7±0.7*	1.3±0.2*	3.1±0.6*†§	7.3±1.2*†‡§	1.4±0.5
AngII 0.5	0.1±0.0	0.3±0.1	0.7±0.1†‡	1.6±0.2†‡§	2.3±0.4†‡§	0.5±0.2†
LSG stim+AngII 0.5	1.3±0.1*	1.7±0.6*	0.9±0.1†	2.8±0.6†§	6.9±1.3*†‡§	1.2±0.6
Group III (n=6)						
Baseline	0.2±0.1	0.3±0.1	0.9±0.1†‡	4.4±0.8†‡§	5.0±0.7†‡§	0.7±0.2†
Ischemia	0.2±0.0	1.4±0.6	16.2±5.7†‡*	12.3±4.0†‡*	14.3±5.4†‡*	0.5±0.1†§
Reperfusion	0.4±0.2	0.4±0.1	0.5±0.1*	1.4±0.2*†‡§	2.3±0.4*†‡§	0.5±0.1
Group IV (n=7)						
Baseline	0.1±0.1	0.2±0.3	0.9±0.3†‡	5.2±0.6†‡§	5.8±0.7†‡§	0.6±0.1†
Ischemia+AngII 0.5	0.2±0.1	0.9±0.2	10.9±3.8†‡*	11.2±3.5†‡*	14.1±3.5†‡*	0.6±0.1†§
Reperfusion	0.4±0.2	0.3±0.1	1.0±0.5	1.7±0.3*†‡	2.2±0.4*†‡§	0.7±0.2

AngII indicates intracoronary infusion of angiotensin II (ng/kg per minute); LSG, left stellate ganglion.

Intracoronary infusion of angiotensin II had no effect on circulatory or interstitial norepinephrine concentrations in any of the experimental protocols. Data are mean±SEM.

*P<0.05 vs baseline; †P<0.05 vs arterial plasma; ‡P<0.05 vs coronary vein; §P<0.05 vs MIF LAD (control); ||P<0.05 desipramine vs desipramine+phentolamine.

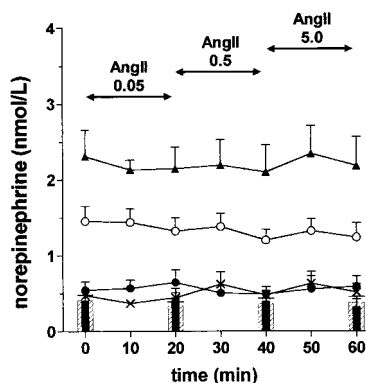


Figure 2. Effect of angiotensin II on basal cardiac sympathetic tone. Data are shown for $NE_{MIF,LAD}$ (●), $NE_{MIF,LAD}$ + desipramine (○), $NE_{MIF,LAD}$ + desipramine + phentolamine (▲), $NE_{MIF,L,CX}$ (×), arterial (solid bars), and coronary venous (hatched bars) norepinephrine concentrations. AngII indicates intracoronary infusion of angiotensin II (0.5 ng/kg per minute). Data are mean \pm SEM, $n=7$.

mained depressed, whereas LV end-diastolic pressure returned to baseline. In addition, heart rate increased and LV dP/dt_{max} decreased. Angiotensin II infusion during ischemia did not alter the hemodynamic response to ischemia and reperfusion. Because LAD flow was kept at 30% of baseline during ischemia, any effect of angiotensin II on LAD flow was prevented.

Norepinephrine Concentrations

At baseline, $NE_{MIF,LAD}$ and $NE_{MIF,L,CX}$ were similar and 3 times the arterial norepinephrine concentration ($P<0.05$; Table 2). Under U1-blockade with desipramine, $NE_{MIF,LAD}$ increased approximately 5-fold, irrespective of the presence of α -adrenoceptor blockade. $NE_{MIF,LAD}$ tripled during the first 20 minutes of ischemia and continued to rise up to 15-fold at 60 minutes of ischemia (Table 2, Figure 4). Under U1-inhibition, the rate of rise of $NE_{MIF,LAD}$ was attenuated, so that, from 40 minutes of ischemia, $NE_{MIF,LAD}$ in the presence of desipramine

TABLE 3. Intracoronary Angiotensin II Infusion and Cardiovascular Function During Sympathetic Activation

	Ang II	Baseline	LSG Stimulation
Mean arterial pressure, mm Hg	–	77 \pm 5	95 \pm 7*
	+	75 \pm 4	93 \pm 6*
Cardiac output, L/min	–	2.5 \pm 0.2	2.8 \pm 0.2*
	+	2.4 \pm 0.1	2.7 \pm 0.1*
Heart rate, bpm	–	114 \pm 4	115 \pm 4
	+	111 \pm 5	114 \pm 4
LV dP/dt_{max} , mm Hg/sec	–	1197 \pm 103	3445 \pm 340*
	+	1172 \pm 102	3383 \pm 233*
LV end diastolic pressure, mm Hg	–	11 \pm 2	9 \pm 2
	+	11 \pm 2	9 \pm 2
LAD flow, mL/min	–	27 \pm 4	36 \pm 4*
	+	27 \pm 4	35 \pm 4*

AngII indicates intracoronary infusion of angiotensin II (0.5 ng/kg per minute).

Data are presented as mean \pm SEM, $n=7$.

* $P<0.05$ vs baseline.

was similar to $NE_{MIF,LAD}$ without desipramine. On reperfusion, $NE_{MIF,LAD}$, $NE_{MIF,LAD}$ in the presence of desipramine, and coronary venous norepinephrine concentrations declined rapidly, with the early rate of decline being most pronounced for $NE_{MIF,LAD}$ without desipramine (Figure 4).

During ischemia, intracoronary infusion of angiotensin II raised angiotensin II concentrations in the LAD from 9 ± 1 to 2323 ± 231 pmol/L, whereas coronary venous concentrations increased from 13 ± 3 to 408 ± 46 pmol/L, indicating that $80 \pm 5\%$ of angiotensin II was extracted over the coronary bed. However, maximum $NE_{MIF,LAD}$ during the 60 minutes of ischemia was not modified by concomitant angiotensin II infusion (Table 2 and Figure 4). Within 120 minutes of reperfusion, $NE_{MIF,LAD}$ and coronary venous norepinephrine concentrations had returned to baseline and were similar for groups III and IV. $NE_{MIF,L,CX}$ and arterial norepinephrine concentrations remained unchanged during the course of the experiment in both groups.

Infarct Size

The 70% LAD flow reduction resulted in an ischemic area (area at risk) that composed $32 \pm 4\%$ of the LV mass in both groups. Infarct size was $37 \pm 7\%$ and $37 \pm 4\%$ of the area at risk in groups III and IV, respectively.

Discussion

This study provides no evidence for facilitation of cardiac norepinephrine release by angiotensin II under various experimental conditions in the intact porcine heart, because intracoronary infusion of angiotensin II did not modulate (1) basal sympathetic tone, (2) exocytotic norepinephrine release during sympathetic activation produced by left stellate ganglion stimulation, or (3) exocytotic and nonexocytotic norepinephrine release during myocardial ischemia.

Intracoronary Angiotensin II Infusion and Basal Cardiac Sympathetic Tone

Although the intracoronary angiotensin II infusions in our experiments caused large increments in coronary venous angiotensin II concentrations, no increments in interstitial or coronary venous norepinephrine concentrations were observed (Table 2). Inhibition of norepinephrine neuronal reuptake by co-perfusion of microdialysis probes with desipramine and inhibition of the presynaptic α_2 -adrenergic receptor-mediated negative feedback of norepinephrine release with phentolamine did not unmask an angiotensin II-mediated increase in $NE_{MIF,LAD}$.

Although our findings agree with studies that also failed to demonstrate an effect of angiotensin II on basal norepinephrine concentration and norepinephrine spillover,^{9–11} they are at variance with other studies that have shown angiotensin II to increase basal sympathetic tone.^{3,4} It could be argued that, in anesthetized animals, facilitation of norepinephrine release by angiotensin II is difficult to demonstrate because of low basal norepinephrine concentrations compared those with awake swine.²⁶ However, Dendorfer and coworkers³ have stated that facilitation of norepinephrine release by angiotensin II is in fact easier to demonstrate when background sympathetic tone is low. An additional possible explanation

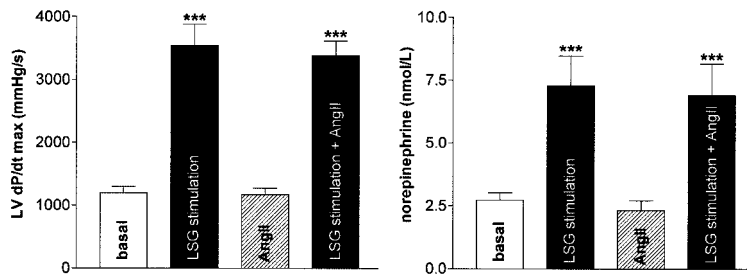


Figure 3. Effect of angiotensin II on LV dP/dt_{max} (left panel) and NE_{MIF,LAD}+desipramine+phentolamine (right panel) during left stellate ganglion (LSG) stimulation. Data are mean±SEM, n=7. ***P<0.001 versus prestimulation values.

for the discrepancy between our results and those of Dendorfer³ and Teisman⁴ is the difference between the dominant sympathetic control of cardiac function in rats and dominant parasympathetic control of cardiac function in pigs and also in humans, which could imply that rat hearts are more sensitive to facilitation of sympathetic activity by angiotensin II.

Angiotensin II may not only facilitate the neuronal release of norepinephrine but may also inhibit its neuronal reuptake.^{18,27} As neuronal reuptake is an important determinant of NE_{MIF} under baseline conditions as well as during increased sympathetic tone,^{19,21} an increase in NE_{MIF} concentration through inhibiting neuronal reuptake by angiotensin II would almost certainly have been detected in this study.

We can also exclude that a putative facilitating effect of angiotensin II on norepinephrine release was masked by a hemodynamically mediated increase in norepinephrine clearance. First, we used intracoronary angiotensin II infusions to prevent significant systemic hemodynamic effects. Second, the tendency of LAD flow to decrease would have favored an increase in NE_{MIF} by blunting norepinephrine clearance. In fact, the angiotensin II-associated increase in norepinephrine observed in some studies might be explained by a decrease in clearance caused by angiotensin II-induced vasoconstriction.⁴

Intracoronary Angiotensin II Infusion and Norepinephrine Release During Sympathetic Activation

In the present study, sympathetic stimulation was induced by electrical stimulation of the left stellate ganglion, resulting in marked increases in LV dP/dt_{max}, blood pressure, and LAD flow. These hemodynamic effects were not enhanced by an intracoronary infusion of angiotensin II (Table 3 and Figure 3). Similarly, ganglion stimulation increased NE_{MIF} up to 5-fold. In the presence of U1- and α-adrenoceptor blockade, the absolute increase in NE_{MIF} was the most substantial, suggesting an important negative feedback mechanism through presynaptic α-adrenergic receptors, most likely of the α₂-subtype. Again, infusion of angiotensin II did not augment this increase, irrespective of the presence of U1- and α-adrenergic receptor blockade (Table 2 and Figure 3). These results are in agreement with other studies that also failed to demonstrate enhanced norepinephrine release by angiotensin II during sympathetic activation in humans with and without chronic heart failure,^{10–12} and in particular with Rundqvist et al,⁸ who demonstrated that intracoronary administration of the angiotensin-converting enzyme (ACE) inhibitor enalaprilat failed to attenuate the increase in cardiac norepinephrine spillover following sympathetic activation. In contrast, other studies using electrical stimulation *in vitro*,^{1,2,5} as well as studies in humans,^{6,7} did demonstrate angiotensin II-induced

TABLE 4. Intracoronary Infusion of Angiotensin II and Cardiovascular Function During Ischemia

	AngII	Baseline	Ischemia (60 min)	Reperfusion (120 min)
Mean arterial pressure, mm Hg	–	95±2	89±1	85±3*
	+	93±3	86±6	79±4*
Cardiac output, L/min	–	2.7±0.2	2.4±0.2*	2.3±0.1*
	+	2.7±0.1	2.3±0.1*	2.0±0.2*
Heart rate, bpm	–	121±4	127±6	136±6*
	+	117±6	124±6	138±10*
LV dP/dt _{max} , mm Hg/sec	–	1994±141	1833±85	1615±104*
	+	1668±111	1554±115	1458±181
LV end diastolic pressure, mm Hg	–	12±2	14±2	12±1
	+	6±2	9±2*	8±2
LAD Flow, mL/min	–	27±4	8±1*	35±6
	+	35±3	10±1*	44±5*

AngII indicates intracoronary infusion of angiotensin II (0.5 ng/kg per minute).

Data are mean±SEM (AngII–, n=6; AngII+, n=7).

*P<0.05 vs baseline.

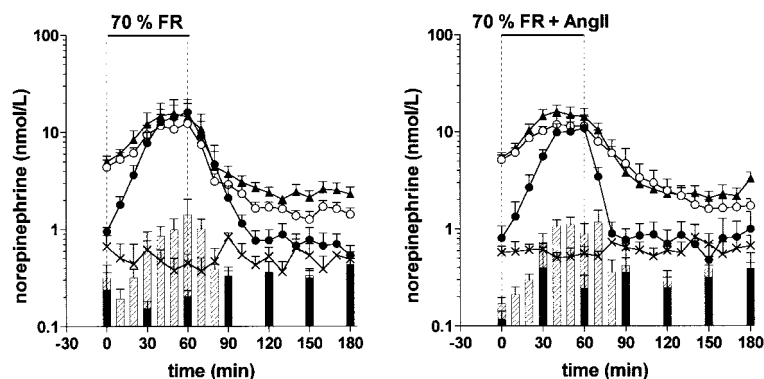


Figure 4. Effect of angiotensin II on the time course of changes in norepinephrine concentrations during 70% LAD flow reduction (70% FR) and reperfusion. Data are shown for $NE_{MIF,LAD}$ (●), $NE_{MIF,LAD}$ + desipramine (○), $NE_{MIF,LAD}$ + desipramine + phentolamine (▲), $NE_{MIF,LCx}$ (×), arterial (solid bars), and coronary venous (hatched bars) norepinephrine concentrations. Data are mean \pm SEM, $n=6$ (left panel) and $n=7$ (right panel).

augmentation of sympathetic activation. Three of these studies were on the heart.^{2,5,7} Of the latter, only the study by Saino and colleagues⁷ investigated augmentation of sympathoneural activation by angiotensin II in the intact (human) heart. However, because they did not measure norepinephrine spillover or norepinephrine concentrations directly but estimated differences in sympathetic activity by comparing responses of coronary blood flow and coronary vascular resistance to the diving and cold-pressure tests, with and without simultaneous intracoronary angiotensin II infusion, it cannot be excluded that vasomotor mechanisms other than α -adrenoceptor-mediated vasoconstriction, as a result of facilitated norepinephrine release, are responsible for the observed hemodynamic responses.

Intracoronary Angiotensin II Infusion and Norepinephrine Release During Ischemia

Because angiotensin II has been reported to enhance either nonexocytotic norepinephrine release via activation of the Na^+/H^+ exchanger^{22,23} or exocytotic release via classic calcium-dependent facilitation,^{1,3} we monitored norepinephrine release in MIF during myocardial ischemia produced by LAD flow reduction, which leads to both exocytotic and nonexocytotic norepinephrine release while still permitting intracoronary infusion of angiotensin II during ischemia. The NE_{MIF} increase during 70% flow reduction (15-fold) was much less than previously described during total occlusion (500-fold),²⁰ not only because ischemia was less severe but also because washout of released norepinephrine is partially preserved during 70% flow reduction. We kept LAD flow constant at 30% of baseline, thereby preventing any potential effects of angiotensin II on flow-induced changes in norepinephrine clearance (Table 4). During flow reduction, concomitant infusion of angiotensin II neither augmented the ischemia-induced increase in NE_{MIF} nor altered its time course (Table 2, Figure 4). Our findings are at variance with the attenuation of ischemia-induced norepinephrine release,^{23,28,29} as well as the decrease in sympathetic activity in heart failure^{18,30,31} by ACE-inhibitors or AT_1 -receptor blockers, which had been reported earlier. Several factors may contribute to these apparent conflicting results. (1) Diffusion limitations for angiotensin II from the bloodstream to the perivascular or myocardial sympathetic nerve terminals could have prevented the infused angiotensin II from reaching the interstitial space and occupying AT_1 receptors.⁸ However,

this is unlikely, as we have previously shown that the cardiac tissue concentration of radiolabeled ^{125}I -angiotensin II during ^{125}I -angiotensin II infusion was 75% of its arterial concentration and that most of this angiotensin II is bound to AT_1 receptors.¹⁴ (2) Although inhibition of the renin-angiotensin system may exert a direct effect on norepinephrine release in chronic heart failure,^{18,30,31} the decrease in plasma norepinephrine concentrations during treatment of heart failure with ACE-inhibitors or AT_1 -receptor blockers might also be due to an improvement of cardiac function. (3) The decrease in sympathetic tone with these agents^{18,23,28–31} might not be mediated through peripheral presynaptic AT_1 receptors, but by other mechanisms. For instance, ACE-inhibitors do not only inhibit angiotensin I to angiotensin II conversion, but also limit bradykinin degradation and stimulate prostaglandin formation. Both bradykinin and prostaglandins have been shown to inhibit norepinephrine release.^{28,32} In addition, the interaction between the renin-angiotensin system and the sympathetic nervous system might be mediated through central AT_1 receptors in the brain.^{33–35} (4) Facilitation of norepinephrine release by presynaptic AT_1 -receptor activation might be counteracted by presynaptic AT_1 receptors, which can inhibit norepinephrine release and are down-regulated in cardiomyocytes of patients with chronic heart failure.^{22,23,36}

Perspectives

In this study in pigs, in which, contrary to rats, cardiac function is predominantly parasympathetically controlled, we did not find evidence for facilitation of cardiac norepinephrine release by exogenous angiotensin II under baseline conditions and during sympathetic activation by either stellate ganglion stimulation or acute ischemia, indicating that angiotensin II is not a local mediator of cardiac sympathetic nerve activity. The design of the study does not exclude facilitation of cardiac norepinephrine release by angiotensin II through stimulation of presynaptic AT_1 receptors at sympathetic nerve terminals under pathological conditions such as hypertension and heart failure. In a previous study, we observed an upregulation of AT_1 receptors in the remodeled hypertrophied myocardium, as well as increased circulatory concentrations of angiotensin II during exercise, 1 to 3 weeks after infarction.²⁶ If angiotensin II facilitates norepinephrine release via stimulation of presynaptic angiotensin II receptors, the latter model may be particularly suited to address this

question, and research to explore this issue is under way. Furthermore, because of intrinsic activation of the renin-angiotensin system, this model is also well suited to explore whether local inhibition of the renin-angiotensin system with AT₁-receptor antagonists is associated with a decrease in myocardial norepinephrine release.

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