Epinephrine in the Heart: Uptake and Release, but No Facilitation of Norepinephrine Release

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Uptake and Release, but No Facilitation of Norepinephrine Release

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Background—Several studies have suggested that epinephrine augments the release of norepinephrine from sympathetic nerve terminals through stimulation of presynaptic receptors, but evidence pertaining to this mechanism in the heart is scarce and conflicting. Using the microdialysis technique in the porcine heart, we investigated whether epinephrine, taken up by and released from cardiac sympathetic nerves, can increase norepinephrine concentrations in myocardial interstitial fluid (NE_{MIF}) under basal conditions and during sympathetic activation.

Methods and Results—During intracoronary epinephrine infusion of 10, 50, and 100 ng/kg per minute under basal conditions, large increments in interstitial (from 0.31±0.05 up to 140±30 nmol/L) and coronary venous (from 0.16±0.08 up to 228±39 nmol/L) epinephrine concentrations were found, but NE_{MIF} did not change. Left stellate ganglion stimulation increased NE_{MIF} from 3.4±0.5 to 8.2±1.5 nmol/L, but again, this increase was not enhanced by concomitant intracoronary epinephrine infusion. Intracoronary infusion of tyramine resulted in a negligible increase in epinephrine concentration in myocardial interstitial fluid (EPI_{MIF}), whereas 30 minutes after infusion of epinephrine an increase of 9.5 nmol/L in EPI_{MIF} was observed, indicating that epinephrine is taken up by and released from cardiac sympathetic neurons. Although 68% to 78% of infused epinephrine was extracted over the heart, the ratio of interstitial to arterial epinephrine concentrations was only ≈20%, increasing to 29% with neuronal reuptake inhibition.

Conclusions—Our findings demonstrate epinephrine release from cardiac sympathetic neurons, but they do not provide evidence that epinephrine augments cardiac sympathoneural norepinephrine release under basal conditions or during sympathetic activation. **(*Circulation*. 2002;106:860-865.)**

Key Words: norepinephrine ■ receptors ■ nervous system, sympathetic ■ heart failure

Several in vitro as well as in vivo studies have suggested that epinephrine (EPI) enhances sympathoneural norepinephrine (NE) release through stimulation of presynaptic α_2-adrenoceptors located at the sympathetic nerve terminals.[1–5] This mechanism would be particularly important in the heart because adrenomedullary activation in conditions such as hypertension and heart failure could contribute to the deterioration of cardiac function through chronically increasing sympathoneural NE release by presynaptic facilitation. Indeed, studies have shown that cardiac EPI is released into the coronary circulation of the heart in conditions such as hypertension and heart failure but also during exercise, at rest with advanced age, and in patients with panic disorders.[1,6,7] Furthermore, we have recently shown that prolonged myocardial ischemia is associated with a progressive increase of EPI concentrations in the myocardial interstitial fluid (EPI_{MIF}).[8] Evidence that such an increased cardiac EPI concentration leads to an increase in cardiac NE by presynaptic facilitation is, however, scarce and conflicting.[1,5,9] In the present study we have tested the hypothesis that locally administered and coreleased EPI modulates interstitial NE (NE_{MIF}) concentrations under basal conditions and during sympathetic activation induced by electrical stimulation of the left stellate ganglion. At the same time we investigated the extent to which EPI is taken up by and released from cardiac sympathetic nerves and the source of cardiac EPI, because it is still unclear whether EPI is released from sympathetic nerve terminals after it has been taken up from the circulation or whether it is released from extraneuronal stores.[10,11]

For this purpose, we measured interstitial EPI and NE concentrations in the intact porcine heart by using the microdialysis technique. The porcine heart is especially suitable as a model for studying the cardiac sympathetic nervous system, as the distribution of β_1/β_2 adrenoceptors (80%/20%)[12] and the prevailing parasympathetic control of cardiac function are very much akin to the human heart. Increases in locally released EPI were obtained by loading the heart with EPI by means of intracoronary EPI infusions. The source of cardiac EPI was investigated through the effect of intracoronary tyramine infusions on interstitial EPI concen-

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TABLE 1. Cardiovascular Function During Intracoronary Epinephrine Infusion

		Intracoronary Infusion of Epinephrine, ng/kg per minute		
	Baseline	10	50	100
Mean arterial pressure, mm Hg	89±4	85±4	77±4*	76±3*
Cardiac output, L/min	2.6±0.2	2.6±0.2	2.8±0.2	3.1±0.3*
Heart rate, bpm	125±7	130±6	137±6*	144±7*
Systemic vascular resistance, mm Hg $min^{-1} \cdot L$	37±4	32±3*	29±3*	26±2*
Stroke volume, mL	21±3	22±2	20±3	27±5
LV dP/dt_{max}, mm Hg/s	1604±136	2483±191*	3068±222*	3716±186*
LV end-diastolic pressure, mm Hg	7±2	6±2	6±2*	6±2*
LAD flow, mL/min	29±4	41±4*	41±4*	43±4*

Values are mean±SEM (n=9).
*$P<0.05$ vs baseline.

trations before and after loading the heart with EPI. Because tyramine only displaces catecholamines from their storage vesicles in the sympathetic nerve terminals after it has been taken up by the neuronal reuptake (U1) mechanism,[13,14] catecholamines released by tyramine are exclusively from neuronal origin. Because >80% of neuronally released NE is taken up by sympathetic nerves of the porcine heart through the U1 mechanism,[13] the U1 inhibitor desipramine was added to the perfusate of one of the microdialysis probes to provide local U1 blockade. In addition, we also accounted for a possible inhibition of NE release through stimulation of presynaptic α_2-adrenoceptors by adding the nonselective α-adrenoceptor antagonist phentolamine to the perfusate of another probe in combination with desipramine.

Methods

Animal Procedures

All experiments were performed in accordance with the "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 x Yorkshire pigs of either sex (30 to 35 kg, n=19; Oude Tonge, the Netherlands) were used. Treatment, surgical procedure, and positioning of catheters and flow probes have been described previously.[8,13] In animals subjected to sympathetic stimulation, the left stellate ganglion was dissected, and an electrode was inserted into the ganglion as described previously by Gootman et al[15] and connected to a nerve stimulator (Grass S9; pulses of 12 V, 10 Hz and 5 ms).

Microdialysis probes were implanted in left ventricular (LV) myocardium: one in the region perfused by the left circumflex coronary artery (LCx) and three in the area perfused by the left anterior descending coronary artery (LAD). One of the LAD probes was coperfused with desipramine (DMI) (Sigma, 100 μmol/L),[14] and one LAD probe was coperfused with DMI and the nonselective α-adrenoceptor blocking agent phentolamine (PHA) (Department of Pharmacy, University Hospital Dijkzigt, Rotterdam; 100 μmol/L). The microdialysis technique, probe characteristics, probe recovery, handling, and analysis of the microdialysis and plasma samples and its sensitivity have been described previously.[13,16]

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 μL/min; dialysate was collected at 10-minute intervals, in which blood was collected from the central aorta (Ao) and the anterior interventricular coronary vein (CV), which drains the LAD perfusion territory. In 9 animals, intracoronary EPI was administered at infusion rates of 10, 50, and 100 ng/kg per minute, each for 30 minutes. Tyramine was infused (26.7 μg/kg per minute) for 30 minutes into the LAD 30 minutes after discontinuation of the EPI infusions. To prevent possible interference of tyramine infusions with subsequent EPI infusions, the effect of tyramine infusion under basal conditions was studied in 4 separate animals. Finally, in 6 animals the left stellate ganglion was stimulated electrically before and during concomitant infusion of 50 ng/kg per minute EPI.

Data Analysis and Calculations

Dialysate EPI and NE concentrations were corrected for probe recovery to yield EPI_{MIF} and NE_{MIF}.[8,13] Lower limits of detection were 0.2 nmol/L in dialysate and 0.02 nmol/L in plasma.[16] Baseline values were determined by averaging the three measurements over the 30-minute period before intervention. EPI plasma concentrations in the LAD (EPI_{CA}) were calculated from EPI infusion rate, coronary plasma flow, and EPI_{Ao}.

In addition, cardiac extraction of EPI, the ratio of the absolute changes of interstitial to the absolute changes of arterial EPI concentrations $\Delta MIF/\Delta CA$ during EPI infusion, the percentage of EPI that can be recovered from the MIF and that is taken up by U1, spillover, uptake of released EPI from the interstitium, neuronal release rate, and efficiency of total uptake of EPI were calculated.[8,13]

Statistical Analysis

All data are expressed as mean±SEM. For statistical analysis, 2-way ANOVA, 1-way ANOVA for repeated measures with Dunnett's multiple comparison test as post hoc test, Student's *t* test, and linear regression analysis were used as appropriate.

Results

Effect of Intracoronary EPI Infusions on NE and EPI Concentrations

The intracoronary EPI infusions caused dose-dependent increases in LV dP/dt_{max} (130%), heart rate (15%), and cardiac output (20%), whereas mean arterial pressure (−15%), systemic vascular resistance (−30%), and LV end-diastolic pressure (−20%) decreased (Table 1). In contrast, LAD flow increased about 45%, independent of the infused dose.

Intracoronary infusion of EPI caused dose-dependent increases in EPI_{CV} from 0.16±0.08 nmol/L at baseline up to 228±39 nmol/L during infusion of 100 ng/kg per minute and $EPI_{MIF,\ LAD}$ from 0.31±0.05 nmol/L up to 140±30 nmol/L (Figure 1). U1 inhibition did not affect $EPI_{MIF,\ LAD}$ at baseline

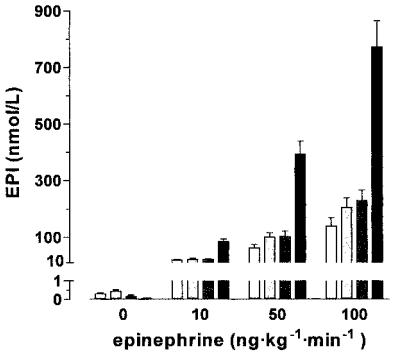

Figure 1. Effect of intracoronary epinephrine infusions on coronary venous and interstitial epinephrine concentrations. Data are shown for $EPI_{MIF,\ LAD}$ (white), $EPI_{MIF,\ LAD}$+DMI (light gray), EPI_{CV} (dark gray), and EPI_{CA} (black bars). Data are mean±SEM, n=9.

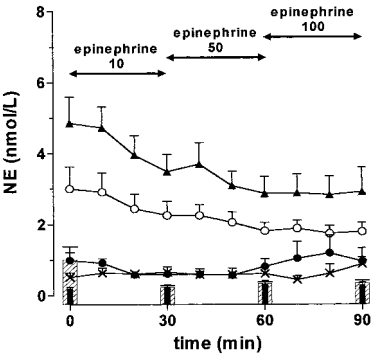

Figure 2. Effect of intracoronary infusion of epinephrine (ng/kg per minute) on basal cardiac sympathetic tone. Data are shown for $NE_{MIF,\ LAD}$ (●), $NE_{MIF,\ LAD}$+DMI (○), $NE_{MIF,\ LAD}$+DMI+PHA (▲), $NE_{MIF,\ LCX}$ (x), NE_{Ao} (solid bars), and NE_{CV} (hatched bars). Data are mean±SEM, n=9.

and the lowest EPI infusion rate but caused an increase in $EPI_{MIF,\ LAD}$ to similar values as EPI_{CV} at the two higher infusion rates. Although the cardiac EPI extraction was 68% to 78%, there was a marked gradient between interstitial and circulatory concentrations. During the intracoronary EPI infusions, the ΔMIF/ΔCA ratio for EPI (Table 2) was 27±4%, 19±3%, and 21±3% in the absence of U1 blockade and 29±3% in the presence of U1 blockade, irrespective of the EPI infusion rate. Despite the large increments in circulatory and interstitial EPI concentrations, the pharmacokinetic parameters for EPI as spillover, rate of uptake, rate of neuronal release, and efficiency of uptake remained unchanged (Table 2). Notwithstanding the aforementioned large increments in EPI_{CV} and $EPI_{MIF,\ LAD}$, $NE_{MIF,\ LAD}$, NE_{CV}, and NE_{Ao} did not change (Figure 2).

Intracoronary EPI Infusion and NE Release During Sympathetic Activation

Left stellate ganglion stimulation caused increases in MAP (21%), LAD flow (36%), and in particular LV dP/dt_{max} (184%, Table 3 and Figure 3) and caused a rise in NE_{MIF}, particularly in the presence of U_1 and α-adrenoceptor blockade, where $NE_{MIF,\ LAD}$ increased from 3.4±0.5 to 8.2±1.5 nmol/L (Figure 3). Although intracoronary infusion of EPI decreased MAP (−25%) and systemic vascular resistance (−27%) and increased HR (22%), LV dP/dt_{max} (93%), and LAD flow (32%), it did not alter the hemodynamic responses to stimulation. Similarly, concomitant EPI infusion did not enhance the NE release on stimulation of the left stellate ganglion (from 2.6±0.3 to 6.9±1.5 nmol/L, Figure 3). In addition, stimulation did not increase $EPI_{MIF,\ LAD}$ (58±8 versus 58±5 nmol/L), whereas EPI_{CV} even decreased (201±16 versus 158±6 nmol/L, $P<0.05$).

Intracoronary EPI Infusion and Tyramine-Induced EPI and NE Release

Intracoronary infusion of tyramine caused increases in mean arterial pressure (20%), heart rate (20%), LV dP/dt_{max} (190%), LAD flow (40%), and $NE_{MIF,\ LAD}$ (12.8±2.9 nmol/L, $P<0.05$). The small increase in heart rate compared with large increase in LV dP/dt_{max} during intracoronary infusion of tyramine can

TABLE 2. Spillover, Uptake, and Release of Epinephrine Compared With Norepinephrine

	Epinephrine, ng/kg per minute			Norepinephrine, ng/kg per minute	
	10	50	100	110	330
ΔMIF/ΔA, %	27±4	19±3	21±3	10±1	11±1
$\Delta MIF_{DMI}/\Delta A$, %	29±4	29±3*	31±3*	21±3*	36±5*†
Fx U1, %	17±5	37±6†	33±7†	51±7	67±5
Extraction, %	78±3	74±5	68±5†	79±4	69±3
SO, pmol/min	2.4±1.3	2.8±1.4	3.7±2.2	35±6	39±5
Ur, pmol/min	46±10	51±14	36±9	194±33	204±51
Rr, pmol/min	49±11	54±14	40±10	229±37	243±53
EffU, %	95±3	96±2	94±3	84±2	79±2

Values are mean±SEM. Epinephrine values are derived from data during intracoronary infusions of epinephrine in the present study. Norepinephrine values are derived from historic data during systemic intravenous infusions of norepinephrine.[8]

ΔMIF/ΔCA indicates the ratio of absolute changes of interstitial to absolute changes of arterial concentrations; Fx U1, percentage recovered from myocardial interstitial fluid that is taken up by Uptake 1; SO, spillover; Ur, uptake of released (nor)epinephrine from the interstitium; Rr, neuronal release rate; and Eff_U, efficiency of total uptake.

*$P<0.05$ vs lowest dose.

†$P<0.05$ vs without DMI.

TABLE 3. Cardiovascular Function During Stellate Ganglion Stimulation Before (−) and During (+) Intracoronary Infusion of Epinephrine

	Baseline	Left Stellate Ganglion Stimulation
Mean arterial pressure, mm Hg		
−	87±3	105±5*
+	67±11†	84±8*†
Cardiac output, L/min		
−	2.6±0.2	2.7±0.3
+	2.8±0.5	3.2±0.5
Heart rate, bpm		
−	108±6	115±7
+	132±4†	137±5†
Systemic vascular resistance, mm Hg · min^{-1} · L		
−	34±3	41±4
+	25±2†	28±3†
Stroke volume, mL		
−	25±1	24±1
+	22±4	24±4
LV dP/dt_{max}, mm Hg/s		
−	1485±36	4220±374*
+	2864±507†	5596±980*†
LV end-diastolic pressure, mm Hg		
−	12±2	10±2
+	8±3	7±3
LAD flow, mL/min		
−	28±5	38±5*
+	37±3†	42±8

Values are mean±SEM (n=6). Epinephrine was given in an intracoronary infusion of 50 ng/kg per minute.

*$P<0.05$ vs baseline.

†$P<0.05$ vs before intracoronary infusion of epinephrine.

be explained by the poor perfusion of the sinus node with tyramine by using this particular route of administration. These responses were not affected by a preceding intracoronary EPI infusion (Figure 4, Table 4). In contrast to the increase in $NE_{MIF,\ LAD}$, the change in $EPI_{MIF,\ LAD}$ during tyramine infusion was negligible before but increased markedly after intracoronary infusion of EPI (9.5±3.0 nmol/L, $P<0.05$). This was also reflected by the tyramine-induced increase in EPI_{CV} from 0.09±0.01 nmol/L before to 8.1±2.7 nmol/L after EPI infusion ($P<0.05$).

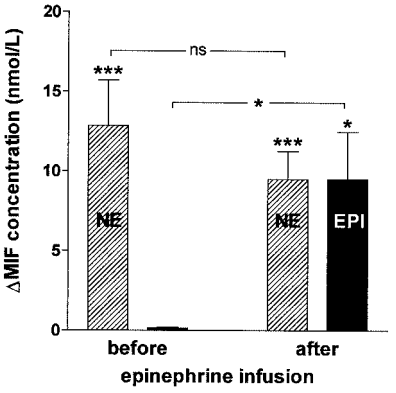

Figure 4. Effect of intracoronary tyramine infusion on concentrations of NE and EPI in MIF before and after intracoronary infusion of epinephrine. Data are mean±SEM, n=9.

Discussion

Effect of Intracoronary EPI on NE Release

Although under basal conditions intracoronary EPI infusions caused a 450-fold increase in interstitial and a 1400-fold increase in coronary vein EPI concentration, we did not detect any changes in interstitial or coronary vein NE concentrations. Even under U1 inhibition and α-adrenoceptor blockade to prevent, respectively, rapid clearance and presynaptic α_2-adrenoceptor–mediated inhibition of NE release by EPI or NE itself, interstitial NE concentration did not increase during intracoronary infusion of EPI.

It could be argued that in anesthetized animals, facilitation of NE release by EPI is difficult to demonstrate because of the low basal NE concentrations as compared with awake swine.[13,17] Hence, we investigated the effects of EPI on NE release during sympathetic activation induced by electrical stimulation of the left stellate ganglion. Left stellate ganglion stimulation resulted in a marked increase in LV dP/dt_{max}, LAD flow, and mean arterial pressure, but these responses were not enhanced by an intracoronary infusion of EPI (Table 2 and Figure 3). During stimulation of the left stellate ganglion, interstitial NE concentration increased up to 5-fold. The absolute increase in $NE_{MIF,\ LAD}$ was most pronounced in the presence of U1 and α-adrenoceptor blockade, underscoring the importance of the α_2-adrenoceptor–mediated feedback mechanism that inhibits neuronal NE release. Similar to the hemodynamic responses, concomitant infusion of EPI did not augment the response of NE to left stellate ganglion stimulation, nor in the presence of U1 inhibition and α-adrenoceptor blockade (Figure 3).

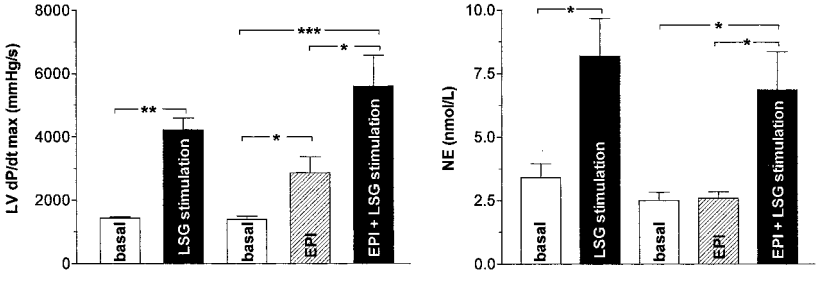

Figure 3. Effect of intracoronary epinephrine infusion on LV dP/dt_{max} (left) and $NE_{MIF,\ LAD}$+DMI+PHA (right) during left stellate ganglion stimulation (LSG). EPI indicates intracoronary infusion of epinephrine (50 ng/kg per minute). Data are mean±SEM, n=6. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

TABLE 4. Cardiovascular Function During Intracoronary Infusion of Tyramine Before (−) and After (+) Intracoronary Infusion of Epinephrine

	Baseline	Tyramine
Mean arterial pressure, mm Hg		
−	90±1	109±4*
+	78±3†	97±4*†
Cardiac output, L/min		
−	2.7±0.3	3.2±0.4*
+	2.5±0.2	3.2±0.1*
Heart rate, bpm		
−	128±7	152±9*
+	128±8	143±9*
Systemic vascular resistance, mm Hg · min^{-1} · L		
−	36±4	37±4
+	32±3	30±2†
Stroke volume, mL		
−	22±2	21±2
+	20±2	24±2
LV dP/dt_{max}, mm Hg/s		
−	1683±89	4819±433*
+	1558±213	4106±333*†
LV end diastolic pressure, mm Hg		
−	6±1	6±3
+	7±1	7±2
LAD flow, mL/min		
−	24±2	34±3*
+	24±3	33±4*

Values are mean±SEM (n=9). Epinephrine was given in 3 consecutive intracoronary infusions, each for 20 minutes (10, 50, and 100 ng/kg per minute). The intracoronary infusion of tyramine was started 30 minutes after the epinephrine infusions were discontinued.

*$P<0.05$ vs baseline.

†$P<0.05$ vs before intracoronary infusion of epinephrine.

Our results are corroborated by studies that also failed to demonstrate enhanced NE release by EPI in other tissues[18–20] and in particular by the findings of Thompson et al,[9] who demonstrated that EPI did not increase NE spillover in the human heart. However, they are at odds with studies showing augmentation of pressor responses,[2,4] increased plasma NE concentrations,[21] and increased forearm NE spillover in humans,[3,22] and in particular with two studies (also about the heart) that reported increased NE outflow in rat atria[5] and in the human heart.[1] The reason for the discrepancy between our findings and those from the study by Majewski et al[5] is unclear but may be related to differences in species (especially because cardiac function in the rat is under predominant sympathetic control unlike that in pigs and humans) and tissue (atria versus ventricle) studied. The other study by Rumantir et al[1] did not investigate the direct effect of EPI on cardiac NE but only provided circumstantial evidence for the epinephrine hypothesis through demonstration of a significant correlation between the respective cardiac spillovers of NE and EPI in hypertensive patients. The latter can also be interpreted as evidence that NE and EPI are coreleased from sympathetic neurons.

With the low-dose infusion of EPI, EPI_{MIF} concentration increased ≈20-fold. This concentration probably is higher than EPI_{MIF} concentrations occurring during congestive heart failure. Nevertheless, we think that this EPI_{MIF} concentration is still in the relevant range as in a previous study in which we showed that EPI_{MIF} concentration rose to similar values during severe ischemia.[8]

It might be argued that in the present study, facilitation of NE release by EPI was obscured by either increased clearance or sympathoinhibition. Indeed, LAD flow increased by 45% during the lowest intracoronary EPI infusion, which might explain the decrease in $NE_{MIF, LAD}$ observed in the presence of α_2-adrenoceptor blockade and U1 inhibition (Figure 2). However, there were no further changes in LAD flow and $NE_{MIF, LAD}$ when EPI infusion rate was further increased 5- to 10-fold (Table 1 and Figure 2). In addition, the absence of an increase in $NE_{MIF, LAD}$ during intracoronary infusion of EPI under U1 inhibition and α-adrenoceptor blockade indicates that the potentially facilitating effect of EPI on NE release was not masked by rapid clearance by U1 and local sympathoneural inhibition. Finally, it could be argued that central sympathoinhibition during the intracoronary infusion of EPI influenced our results. This is unlikely, however, because the decrease in blood pressure that occurred during EPI infusion would promote an increase rather than a decrease in sympathetic outflow, although the absence of an increase in sympathetic activity probably occurred because of the pentobarbital anesthesia.[23]

EPI in the Heart: Release and Uptake

Under basal conditions, the tyramine-induced EPI release could not be demonstrated, which is in agreement with earlier results in the intact rabbit heart[24] and probably reflects the low intraneuronal EPI content (1% to 2% of cardiac NE concentrations).[25] After loading the heart with EPI by means of an intracoronary EPI infusion, tyramine caused substantial increases in EPI_{MIF} and EPI_{CV}, which were comparable to the increases in NE_{MIF} and NE_{CV}. These findings unequivocally demonstrate that in the porcine heart, EPI can be taken up from the circulation by and released from the sympathetic nerve terminals.

We found an EPI extraction of ≈70% for the porcine heart. Other experimental and human studies have reported that the extraction of arterially delivered EPI by the myocardium during a single pass is ≈50%. In all cases, however, the cardiac extraction of EPI is considerably lower than the cardiac extraction of NE (70% to 85%).[1,6,13,26–28] Because U1 is the major determinant of the cardiac clearance of catecholamines and the affinity of EPI for the U1 mechanism is lower than that of NE,[27] it is likely that the difference in extraction originates from this difference in affinity for U1. This is also substantiated by the effect of U1 inhibition on ΔMIF/ΔCA. Thus, depending on the infusion rate, only 17% to 37% of infused EPI appears to be cleared by U1, whereas we have previously shown that in the porcine heart U1 clears 51% to 67% of arterially delivered NE.[13] The considerably higher EPI extraction over the porcine heart compared with the human heart, despite the relatively low clearance of EPI by

U1, suggests the presence of a more active extraneuronal clearance mechanism for EPI in the porcine heart, as was also reported for NE.[13] As the resultant of release and uptake, the modest EPI spillover rate of ≈3.0 pmol/min is in close agreement with that estimated for the human heart and is ≈10 times lower than the spillover rate of NE.[1,7,27,28]

In summary, although the present study shows that EPI is taken up by and released from cardiac sympathetic nerves, our findings in the porcine heart do not support the concept that myocardial NE release is facilitated by EPI either under basal conditions or during activation of cardiac sympathetic tone induced by left stellate ganglion stimulation. Hence, we hypothesize that the uptake of EPI by the heart is principally a mechanism for rapid clearance of circulatory EPI and that the small amount of locally released cardiac EPI does not affect cardiac function.

Acknowledgments

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