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Time Course and Mechanism of Myocardial Catecholamine Release During Transient Ischemia In Vivo

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Background—Elevated concentrations of norepinephrine (NE) have been observed in ischemic myocardium. We investigated the magnitude and mechanism of catecholamine release in the myocardial interstitial fluid (MIF) during ischemia and reperfusion in vivo through the use of microdialysis.

Methods and Results—In 9 anesthetized pigs, interstitial catecholamine concentrations were measured in the perfusion areas of the left anterior descending coronary artery (LAD) and the left circumflex coronary artery. After stabilization, the LAD was occluded for 60 minutes and reperfused for 150 minutes. During the final 30 minutes, tyramine ($154 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused into the LAD. During LAD occlusion, MIF NE concentrations in the ischemic region increased progressively from 1.0 ± 0.1 to $524 \pm 125 \text{ nmol/L}$. MIF concentrations of dopamine and epinephrine rose from 0.4 ± 0.1 to $43.9 \pm 9.5 \text{ nmol/L}$ and from <0.2 (detection limit) to $4.7 \pm 0.7 \text{ nmol/L}$, respectively. Local uptake-1 blockade attenuated release of all 3 catecholamines by $>50\%$. During reperfusion, MIF catecholamine concentrations returned to baseline within 120 minutes. At that time, the tyramine-induced NE release was similar to that seen in nonischemic control animals despite massive infarction. Arterial and MIF catecholamine concentrations in the left circumflex coronary artery region remained unchanged.

Conclusions—Myocardial ischemia is associated with a pronounced increase of MIF catecholamines, which is at least in part mediated by a reversed neuronal reuptake mechanism. The increase of MIF epinephrine implies a (probably neuronal) cardiac source, whereas the preserved catecholamine response to tyramine in postischemic necrotic myocardium indicates functional integrity of sympathetic nerve terminals. (*Circulation*. 2000;101:2645-2650.)

Key Words: nervous system, autonomic ■ myocardial infarction ■ microdialysis

Myocardial ischemia is associated with a marked accumulation of norepinephrine (NE) in ischemic tissue.¹⁻⁴ In vitro studies suggest that this is caused by nonexocytotic release of NE from cardiac sympathetic nerves.⁵⁻⁷ In contrast to the normally occurring exocytotic NE release, this nonexocytotic NE release is (1) calcium independent, (2) not under influence of local or central sympathetic stimulation, and (3) not affected by presynaptic inhibition.⁷ Interestingly, these in vitro studies in the sympathetically dominant rat heart also suggest that the ischemia-induced nonexocytotic NE release can be attenuated by neuronal uptake-1 (U1) blockade, indicating that under ischemic conditions, the U1 mechanism is reversed and can operate as a carrier for outward instead of inward NE transport.^{5,6} However, this has not been investigated in parasympathetically dominant human and porcine hearts in vivo. Furthermore, little is known about the myocardial release of epinephrine (E) and dopamine (DA) in the ischemic heart in vivo; this is of particular interest because cardiac E release has been reported in in vitro

studies and in healthy elderly men and patients with severe congestive heart failure at rest and during exercise.⁸⁻¹¹

Microdialysis allows the measurement of catecholamine concentrations in the myocardial interstitial fluid (MIF) in vivo and the investigation of the mechanisms that underlie their local release and clearance.¹² Using an in vivo porcine model, we investigated the time course and magnitude of changes in MIF concentrations of catecholamines during severe myocardial ischemia and reperfusion. To determine the contribution of reversal of the U1 mechanism to ischemia-induced NE release, 1 of the microdialysis probes in the ischemic myocardium was coperfused with the U1 inhibitor desipramine (DMI).^{12,13}

We also determined whether sympathetic nerve endings are functionally impaired during reperfusion after severe myocardial ischemia, as has been suggested for the isolated rat hearts.² For this purpose, local NE response to an intracoronary infusion of tyramine in the postischemic myocardium was compared with the response observed in the

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nonischemic porcine myocardium of control animals previously studied under similar experimental conditions.¹² Tyramine is taken up via U1 into the sympathetic nerve endings, where it releases NE. Tyramine thus provides information on the NE content as well as on the U1 function of sympathetic nerve endings.¹²

Methods

Animal Care

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 Erasmus University Rotterdam.

Surgical Procedure

After an overnight fast, crossbred Landrace×Yorkshire pigs of either sex (weight 30 to 35 kg, n=9) were sedated with ketamine (20 to 25 mg/kg IM), anesthetized with sodium pentobarbital (20 mg/kg IV), intubated, and connected to a respirator for intermittent positive pressure ventilation with a mixture of oxygen and nitrogen. Respiratory rate and tidal volume were set to keep arterial blood gases within the normal range.^{12,14}

Catheters were positioned in the superior caval vein for the continuous administration of sodium pentobarbital (10 to 15 mg · kg⁻¹ · h⁻¹) and saline to replace blood withdrawn during sampling. In the descending aorta, a fluid-filled catheter was placed to monitor aortic blood pressure and blood sampling. Through a carotid artery, a micromanometer-tipped catheter (B. Braun Medical BV) was inserted into the left ventricle (LV) for the measurement of LV pressure and its first derivative, LV dP/dt. After the administration of pancuronium bromide (4 mg), a midsternal thoracotomy was performed, and the heart was suspended in a pericardial cradle. An electromagnetic flow probe (Skalar) was then placed around the ascending aorta for the measurement of cardiac output. After a Doppler flow probe was placed on a proximal segment of the left anterior descending coronary artery (LAD), a cannula (outer diameter=1.3 mm) was inserted distal to this site into the LAD for the administration of tyramine.

Microdialysis probes were implanted in the LV myocardium through the use of a steel guiding needle and split plastic tubing: 1 probe in the region perfused by the left circumflex coronary artery (LCx) and 2 probes in the area perfused by the LAD. To achieve local U1 inhibition, 1 of the LAD probes was coperfused with DMI (100 μmol/L).¹³ In addition, a microdialysis probe was placed in the interventricular coronary vein that drains the LAD region.¹⁵

Dialysis Methodology

The polycarbonate dialysis membrane of the microdialysis probes (CMA/20; Carnegie Medicine AB) has a cutoff value of 20 kDa, a length of 10 mm, and a diameter of 0.5 mm. Probes were perfused with an isotonic Ringer's solution at a rate of 2 μL/min with a CMA/100 microinjection pump. Dialysate volumes of 20 μL (sampling time 10 minutes) were collected in microvials containing 20 μL of a solution of 2% (wt/vol) EDTA and 30 nmol/L *l*-erythro- α -methyl-NE (AMN) as internal standard in 0.08 N acetic acid. Sampling started immediately after insertion of the probes. Plasma samples were drawn into chilled heparin-containing tubes containing 12 mg glutathione. Microdialysis and plasma samples were stored at -80°C until analysis within the next 5 days.^{12,16}

In vivo probe recovery of NE (52±1%) has been determined through retrodialysis with AMN as a calibrator and direct comparison of hemomicrodialysis and plasma samples.^{12,17} In vivo probe recovery for E (68±3%) was determined through a comparison of E concentrations in arterial plasma with E concentrations in the dialysate obtained from the carotid artery probe. The in vivo probe recovery for DA was not determined directly but was assumed to be

similar to the probe recovery of NE because of the similarities of NE and DA in size and charge distribution.

Experimental Protocol

After a 120-minute stabilization period,¹² baseline measurements were obtained during a 30-minute period before the LAD was occluded distal to the first diagonal branch for 60 minutes, with an atraumatic clip, and then reperfused for 150 minutes. During the final 30 minutes of reperfusion, tyramine (154 nmol · kg⁻¹ · min⁻¹) was infused directly into the LAD. At the end of the experiment, the perfusion area of the LAD was determined with an intra-atrial infusion of 30 mL of a 5% (wt/wt) solution of fluorescein sodium during reocclusion of the LAD. During occlusion, ventricular arrhythmias were counted and distinguished as premature ventricular contractions, ventricular tachycardia, or ventricular fibrillation.¹⁸ After the induction of ventricular fibrillation with a 9-V battery, the heart was excised and infarct size was determined with the use of *para*-nitro blue tetrazolium.¹⁴

Analytical Procedures

Plasma catecholamines were determined through HPLC with fluorometric detection after liquid-liquid extraction and derivatization with the fluorogenic agent 1,2-diphenyl-ethylenediamine.¹⁹ For microdialysis samples, the catecholamines are not extracted before fluorometric detection with HPLC but instead were directly derivatized according to the procedure described by Alberts et al.¹⁷ This method suppresses the interference of sulfhydryl compounds on derivatization, thus improving sensitivity.

Reagents and Pharmaceuticals

Ketamine and sodium pentobarbital were obtained from Apharmo BV. Pancuronium bromide was obtained from Organon Teknica BV. Ringer's solution was purchased from Baxter. Tyramine was obtained from the Department of Pharmacy, University Hospital Rotterdam. Fluorescein sodium, *para*-nitro blue tetrazolium, DMI, NE, E, DA, and AMN were purchased from Sigma Chemical Co. EDTA was purchased from Merck. L-Glutathione was obtained from Fluka. Acetic acid was obtained from Baker. 1,2-Diphenyl-ethylenediamine was prepared as reported previously.¹⁹

Statistical Analysis

Five of the 9 animals experienced ventricular fibrillation during LAD occlusion (between 10 and 30 minutes of ischemia) but were successfully defibrillated within 1 minute with the use of 20- to 30-W countershocks and therefore were included in the analysis. Because there were no differences in the hemodynamic and catecholamine responses between animals that fibrillated and the animals that maintained sinus rhythm, the data for all 9 animals were pooled. Catecholamine concentrations obtained with microdialysis were corrected for probe recovery. Lower limits of detection for catecholamines measured with microdialysis and those measured in arterial plasma were 0.2 and 0.02 nmol/L, respectively. Baseline values were determined by averaging the 3 measurements during the 30-minute period before occlusion.¹² Results 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, and Student's *t* tests were used as appropriate.

Results

Systemic Hemodynamics During Ischemia and Reperfusion

Baseline hemodynamic data and the changes produced with 60-minute LAD occlusion and 120 minutes of reperfusion (Table 1) are in accordance with previously published data from our laboratory.¹⁴

TABLE 1. Cardiac and Systemic Hemodynamics at Baseline, at 60 Min of Ischemia, and at 120 Min of Reperfusion

	Baseline	Ischemia 60 Min	Reperfusion 120 Min
Mean arterial pressure, mm Hg	97±2	82±2*	79±5
Cardiac output, L/min	2.4±0.2	2.1±0.1	1.8±0.1*
Heart rate, bpm	114±4	118±7	137±11*
Systemic vascular resistance, mm Hg · min ⁻¹ · L ⁻¹	42±4	41±4	43±5
Stroke volume, mL	22±2	18±1*	15±2*
LV dP/dt _{max} , mm Hg/s	1680±100	1460±110	1380±150
Left ventricular end-diastolic pressure, mm Hg	6.6±0.8	9.3±1.4	10.0±1.9*
LAD flow, mL/min	34±3	0	46±8

Values are given as mean±SEM (n=9).

*P<0.05 vs baseline.

Infarct Size

The LAD occlusion resulted in an ischemic area (area at risk) that composed 29±2% of the LV mass. Infarct size determined at the end of reperfusion was 84±4% of the area at risk.

Catecholamine Concentrations During Ischemia and Reperfusion

At baseline, NE concentrations in MIF (NE_{MIF}) in the LAD and LCx regions were similar to concentrations in the coronary vein (NE_{CV}) but were 3 times the concentrations in arterial plasma (NE_{art}) (P<0.05; Table 2). DA concentrations followed a similar pattern, whereas E was detectable only in arterial plasma. Under U1 blockade, NE_{MIF} increased 5-fold, whereas DA_{MIF} did not change and E_{MIF} remained undetectable.

During the first 10 minutes of ischemia, NE_{MIF} in the LAD region tripled and continued to rise progressively, so

NE_{MIF} increased 500-fold by the end of ischemia (Table 2, Figure 1). In the presence of U1 blockade, the rate of rise of NE_{MIF} was attenuated so that after 20 minutes of ischemia NE_{MIF} under U1 blockade was similar to, and at 60 minutes was less than half of NE_{MIF} in the absence of U1 blockade. NE_{CV} increased progressively to 100-fold its baseline value. On reperfusion, NE_{MIF} under U1 blockade and NE_{CV} declined rapidly, with the early rate of decline being most pronounced for NE_{MIF} in the absence of U1 blockade (Figure 1). Within 120 minutes of reperfusion, catecholamine concentrations in MIF and coronary vein had returned to baseline values. NE_{MIF} in the LCx perfused area and NE_{art} remained unchanged during the course of the experiment.

In the LAD region, E_{MIF} and DA_{MIF} in the absence and presence of U1 blockade and E_{CV} and DA_{CV} followed qualitatively similar patterns as NE, but absolute increments during ischemia were substantially less pronounced (Table 2,

TABLE 2. Circulatory and Interstitial Catecholamine Concentrations at Baseline, at 60 Min of Ischemia, and at 120 Min of Reperfusion

	Arterial Plasma, nmol/L	Coronary Vein, nmol/L	MIF LAD, nmol/L	MIF LAD+DMI, nmol/L	MIF LCx, nmol/L
NE					
Baseline	0.30±0.08	0.86±0.22†	0.97±0.11†	5.76±0.77†‡§	0.96±0.13†
Ischemia	0.32±0.07	90±36*†	524±125*†‡	198±46*†‡§	0.79±0.27†‡§
Reperfusion	0.54±0.23	0.62±0.30	0.54±0.11	1.99±0.28*†‡§	0.62±0.16
E					
Baseline	0.04±0.01	<0.20	<0.20	<0.20	<0.20
Ischemia	0.03±0.01	2.55±1.07*†	4.74±0.71*†	2.09±0.43*†§	<0.20†§
Reperfusion	0.07±0.02	<0.20	<0.20	<0.20	<0.20
DA					
Baseline	0.06±0.01	0.71±0.45†	0.40±0.12†	0.57±0.16†	0.59±0.27†
Ischemia	0.06±0.01	11.6±4.3*†	43.9±9.5*†‡	16.5±5.6*†§	0.47±0.04†‡§
Reperfusion	0.15±0.07	0.27±0.11	0.30±0.10†	0.34±0.13†	0.21±0.03

Values are given as mean±SEM (n=9).

*P<0.05 vs baseline.

†P<0.05 vs arterial plasma.

‡P<0.05 vs coronary vein.

§P<0.05 vs MIF LADCA.

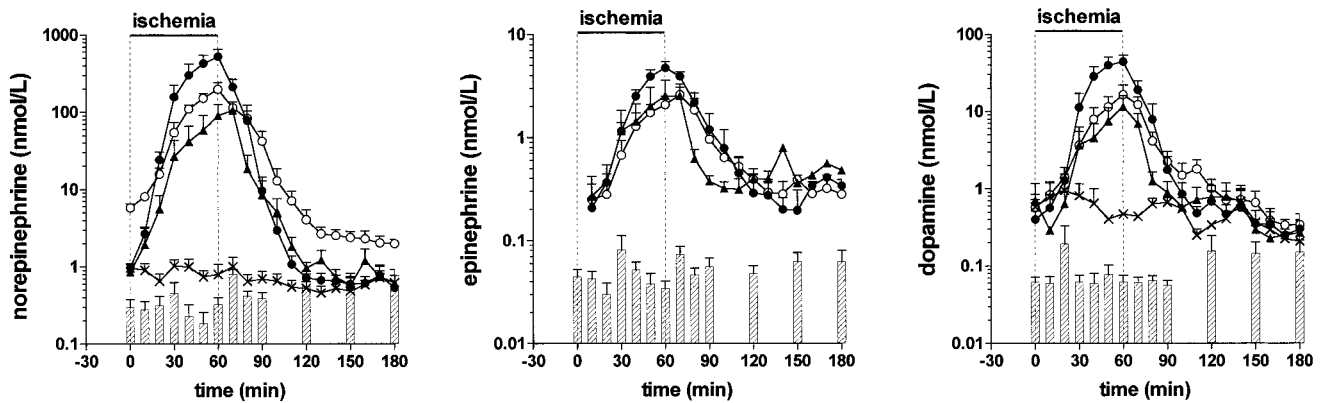


Figure 1. Time course of changes in catecholamine concentrations during ischemia and reperfusion. Data are shown for MIF in LAD region in absence (●) and presence (○) of U1 blockade, MIF in LCx region in absence of U1 blockade (×), and concentrations in arterial plasma (hatched bars) and in coronary vein (▲). Data are given as mean±SEM (n=9).

Figure 1). Again, E_{art} , DA_{art} , and DA_{MIF} in the LCx region did not change over the course of the experiment, whereas E_{MIF} in the LCx region remained undetectable.

Ventricular Arrhythmias During Ischemia

Most of the ventricular arrhythmias occurred within the first 30 minutes of ischemia. The incidence of premature ventricular contractions was particularly high between 20 to 30 minutes of ischemia (a total of 661 and a mean of 73 ± 19 per animal). Five animals experienced ventricular fibrillation but were defibrillated successfully within 1 minute. There was no correlation between NE_{MIF} concentrations and the occurrence of ventricular arrhythmias in general or ventricular fibrillation in particular.

Postischemic Catecholamine Release With Tyramine

Figure 2 shows that the infusion of tyramine directly into the LAD after 120 minutes of reperfusion caused an increase in NE_{MIF} in the postischemic myocardium from 0.6 ± 0.1 to 11.5 ± 1.9 nmol/L ($P < 0.05$). This increment was not different from the increase of from 0.9 ± 0.2 to 13.4 ± 3.2 nmol/L in the LAD region of the normal (nonischemic) swine heart.¹² Furthermore, compared with the increment seen in the ab-

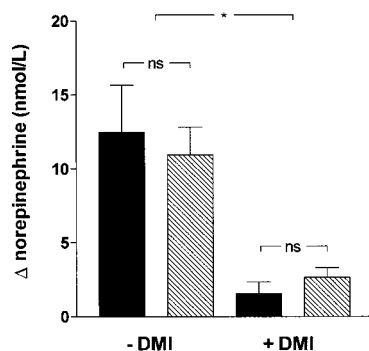


Figure 2. Tyramine-induced NE release in perfusion area of LAD with and without U1 inhibition. Data are shown for postischemic myocardium (n=4, hatched bars) and for historic nonischemic control animals (n=4, solid bars).¹² Data are given as mean±SEM (n=4). * $P < 0.05$.

sence of U1 blockade, the increase in the presence of U1 blockade was minimal.

Discussion

The results of the present study demonstrate that myocardial ischemia is associated with a rapid and massive increase in the concentration of all 3 endogenous catecholamines (NE, E, and DA) in the myocardial interstitial fluid as measured with the microdialysis technique in vivo. As suggested for NE in in vitro studies,^{5,6} the reversed U1 mechanism plays an important role in the release of all 3 catecholamines during ischemia in vivo. Furthermore, our study shows that after 60 minutes of ischemia, which results in massive infarction of the jeopardized myocardium, the functional integrity of sympathetic nerve terminals remains intact. Finally, our results suggest that cardiac interstitial E has a neuronal origin.

Interstitial Catecholamine Concentrations During Basal Conditions

The present study confirms that at baseline, NE_{MIF} is ≈ 3 times the NE_{art} and increases ≈ 6 -fold in response to U1 blockade,^{1,12,20} whereas E_{MIF} was below the detection limit regardless of the presence of U1 inhibition. DA_{MIF} did not rise under U1 blockade, suggesting that U1 does not play a predominant role in the clearance of DA from the interstitial compartment under baseline conditions in the heart. Little is known about the affinity of DA for U1 and the relevance of U1 to DA clearance in the heart. Because the main purpose of the U1 mechanism is to modulate synaptic transmission, it is hard to envisage a substantial role for U1 in the clearance of DA in the absence of any cardiac dopaminergic synaptic transmission. Furthermore, in tissues with known dopaminergic transmission, like brain and kidney, DA is taken up by a specific DA neuronal uptake mechanism that does not take up NE and is poorly inhibited by DMI.^{21,22}

Interstitial Catecholamine Concentrations During Ischemia and Reperfusion

In in vitro experiments in isolated rat hearts, 3 phases of ischemia-induced release of NE, each with a different mechanism, have been recognized.^{2,5,6,23} During the early phase of ischemia (0 to 10 minutes), the release of NE, if present, is

exocytotic and depends on the activation of efferent sympathetic neurons. Accumulation of catecholamines in the extracellular space during this early phase is prevented by the highly efficient U1 mechanism and by presynaptic inhibition by adenosine, which accumulates in cardiac tissue during this phase of ischemia. The latter has been shown to be of particular importance in the rat, because adenosine concentrations are considerably higher than those in other species.²⁴ During the second phase of ischemia (10 to 40 minutes), the release of NE becomes nonexocytotic and is thought to involve the U1 mechanism in the carrier-mediated efflux of NE in reverse of its normal transport direction.^{5,6} During the third phase (>40 minutes ischemia), the release of NE is no longer attenuated by U1 inhibitors, which is explained by the occurrence of structural changes in the neuronal membrane of the myocardial neurons.²

In the present study, a rapid and pronounced increase in MIF concentrations of all 3 catecholamines was observed shortly after occlusion of the LAD. Because released NE is avidly taken up by the cardiac U1 mechanism, we expected a larger rise in NE_{MIF} in the presence of the U1 inhibitor DMI than without U1 inhibition. However, during this first 10 minutes of ischemia, the increment of NE_{MIF} with U1 blockade (5.8 to 8.5 nmol/L) was similar to the increment without U1 blockade (0.9 to 2.7 nmol/L). Possibly, in the parasympathetically dominant porcine heart, U1 carrier-mediated nonexocytotic NE efflux already occurred within the initial 10 minutes of ischemia, so in the presence of DMI, any decrease in U1-mediated clearance was compensated for by a decrease in the ischemia-induced U1 carrier-mediated NE efflux. As mentioned, myocardial release of NE in first 10 minutes of ischemia is not an invariable finding. For example, stimulation-evoked NE release has shown to be suppressed in rat hearts and human atrial tissue but to be facilitated in guinea pig hearts.²⁵

Throughout the ischemic period, MIF catecholamine concentrations rose progressively in the ischemic area. Concentrations of catecholamines did not change in either the nonischemic LCx area or the systemic circulation. The reversal of the U1 mechanism continued to contribute to the catecholamine release during the entire period of ischemia. Thus, U1 blockade attenuated the release of all catecholamines by >50%, indicating that despite infarction of 83% of the area at risk, U1 was operative after 60 minutes of ischemia. Our findings vary from those obtained in the ischemic myocardium of the isolated rat heart, where the reversed U1 mechanism no longer contributes to the release of catecholamines 40 minutes after the induction of ischemia. This difference may be explained by the differences in experimental conditions (eg, in vivo versus in vitro studies) and the species investigated.²

On reperfusion, MIF catecholamine concentrations rapidly declined in the postischemic myocardium. Washout probably was the predominant factor in the clearance of catecholamines in this early phase of reperfusion. However, the decline in the first 10 minutes of reperfusion was substantially greater without inhibition of the U1 mechanism, indicating that the U1 mechanism also contributed significantly to the clearance of NE during early reperfusion. Although it

should be noted that in contrast to techniques used in in vitro studies,^{2,25} the time resolution of the MD technique as presently used does not allow conclusions to be made regarding minute-to-minute changes in catecholamine concentrations. Compatible with previous findings that E and DA are less avidly taken up by U1 than NE,²⁶ the decline in E_{MIF} and DA_{MIF} during reperfusion was not affected by U1 blockade.

Origin of Myocardial Interstitial E

An interesting finding was the ischemia-induced increase in E_{MIF} , albeit small compared with the increase in NE_{MIF} . Because the concentrations of E_{art} and E_{MIF} in the nonischemic LCx region did not change during ischemia, this increase must have originated from the heart. It is currently unclear whether this source is neuronal or extraneuronal. Evidence favoring extraneuronal synthesis and release of E is the presence of the enzyme phenylethanolamine N-methyltransferase in extraneuronal myocardial tissue.^{10,27} Furthermore, an intrinsic cardiac adrenergic cell type outside the sympathetic nervous system, capable of releasing E and NE, has been identified in the human heart.¹⁰ Finally, enhanced cardiac E spillover into the coronary circulation of patients with heart failure during sympathetic stimulation was disproportionate to the spillover of NE, suggesting that E may in part be derived from sources other than chromaffin cells or sympathetic nerves.^{9,11} On the other hand, it is known that sympathetic neurons can take up E from the circulation and release it upon stimulation.²⁸ In the present study, the pattern of release and clearance of E during ischemia and reperfusion was similar to that of NE and DA. Furthermore, inhibition of the neuronal U1 mechanism attenuated the ischemia-induced release of all catecholamines to a similar degree, suggesting a common source, thus favoring a neuronal origin.

Functional Integrity of Sympathetic Nerve Endings

The effects of U1 blockade on the NE_{MIF} responses to ischemia and reperfusion suggested that U1 mechanism of the sympathetic nerves was still functioning during and after the 60-minute LAD occlusion. This is further substantiated by the NE_{MIF} response to tyramine at the end of reperfusion. Similar to NE, tyramine is taken up by neurons through U1, where it displaces NE from the nerve terminals because of its higher affinity for the neuronal storage proteins. Consequently, the tyramine-induced NE release reflects both neuronal NE content and the efficacy of U1.^{12,29} The increase in NE_{MIF} in the postischemic LAD region was very similar to that in the nonischemic control hearts (Figure 2).¹² These findings are in line with those reported by Shindo et al,⁴ who studied the tyramine-induced NE release in nonischemic and postischemic areas in feline hearts after 40 minutes of reperfusion after 40 minutes of ischemia. In addition, in the present study, the attenuation of the tyramine-induced NE release by U1 inhibition in both postischemic and nonischemic groups also was similar (Figure 2). Although the present experimental setup does not allow for any predictions concerning the long-term survival of sympathetic nerves, the present findings indicate that sympathetic nerve terminals remained functionally intact at least during the first few

hours after reperfusion. However, functional alterations of the somata of the sympathetic nerves cannot be entirely excluded. Thus, in a canine model of tachycardia-induced heart failure, impairment of the myocardial contractile response to electrical or chemical stimulation of sympathetic somata was observed at a time when the contractile response to tyramine was completely preserved.³⁰

Implications

Although the pathophysiological significance of the massive accumulation of catecholamines in the ischemic myocardial tissue was not investigated in the present study, there is evidence from experimental as well as clinical studies that high catecholamine concentrations are deleterious to the heart.^{31–34} Several studies have demonstrated NE-dependent antiarrhythmic effects of U1 inhibition during ischemia with either tricyclic antidepressant agents like desipramine and imipramine or structurally unrelated U1 inhibitors like cocaine and nisoxetine.^{7,35} This study provides a possible explanation for this beneficial effect by demonstrating that the reversed U1 mechanism contributes substantially to the release of catecholamines during ischemia.

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