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Cardioprotection in Pigs by Exogenous Norepinephrine but not by Cerebral Ischemia–Induced Release of Endogenous Norepinephrine

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Background and Purpose—Endogenous norepinephrine release induced by cerebral ischemia may lead to small areas of necrosis in normal hearts. Conversely, norepinephrine may be one of the mediators that limit myocardial infarct size by ischemic preconditioning. Because brief ischemia in kidneys or skeletal muscle limits infarct size produced by coronary artery occlusion, we investigated whether cardiac norepinephrine release during transient cerebral ischemia also elicits remote myocardial preconditioning.

Methods—Forty-one crossbred pigs of either sex were assigned to 1 of 7 experimental groups, of which in 6 groups myocardial infarct size was determined after a 60-minute coronary occlusion and 120 minutes of reperfusion. One group served as control (no pretreatment), while the other groups were pretreated with either cerebral ischemia or an intracoronary infusion of norepinephrine.

Results—In 10 anesthetized control pigs, infarct size was 84±3% (mean±SEM) of the area at risk after a 60-minute coronary occlusion and 120 minutes of reperfusion. Intracoronary infusion of 0.03 nmol/kg · min^{-1} norepinephrine for 10 minutes before coronary occlusion did not affect infarct size (80±3%; n=6), whereas infusion of 0.12 nmol/kg · min^{-1} limited infarct size (65±2%; n=7; $P<0.05$). Neither 10-minute (n=5) nor 30-minute (n=6) cerebral ischemia produced by elevation of intracranial pressure before coronary occlusion affected infarct size (83±4% and 82±3%, respectively). Myocardial interstitial norepinephrine levels tripled during cerebral ischemia and during low-dose norepinephrine but increased 10-fold during high-dose norepinephrine. Norepinephrine levels increased progressively up to 500-fold in the area at risk during the 60-minute coronary occlusion, independent of the pretreatment, while norepinephrine levels remained unchanged in adjacent nonischemic myocardium and arterial plasma.

Conclusions—Cerebral ischemia preceding a coronary occlusion did not modify infarct size, which is likely related to the modest increase in myocardial norepinephrine levels during cerebral ischemia. The infarct size limitation by high-dose exogenous norepinephrine is not associated with blunting of the ischemia-induced increase in myocardial interstitial norepinephrine levels. **(*Stroke*. 2001;32:767-774.)**

Key Words: cerebral ischemia, global ■ intracranial pressure ■ myocardial infarction ■ norepinephrine ■ pigs

Ischemic preconditioning, originally described for the myocardium,[1] also occurs in kidney,[2] skeletal muscle,[3] lung,[4] and brain.[5] Przyklenk et al[6] showed that brief regional myocardial ischemia protects not only the jeopardized myocardium during a subsequent coronary artery occlusion but also the adjacent "virgin" myocardium. Furthermore, it has been shown that brief ischemia in remote organs such as kidney,[7] small intestine,[7] and skeletal muscle[8] is also capable of limiting myocardial infarct size produced by a prolonged coronary artery occlusion. Norepinephrine is one of the mediators involved in the signaling pathway leading to ischemic preconditioning,[9,10] and because cerebral ischemia causes a profound release of norepinephrine from sympathetic nerve endings in normal myocardium,[11] this raises the question of whether transient cerebral ischemia before a coronary artery occlusion may also be cardioprotective. In addition, exogenous administration of norepinephrine before a coronary artery occlusion elicits cardioprotection in rabbits[12] and rats.[13] The major aim of this study was therefore to investigate the effect of cerebral ischemia on myocardial infarct size produced by a coronary artery occlusion in pigs. Because the cardioprotective effect of norepinephrine has not been established in pigs, we first studied whether intracoronary infusions of norepinephrine are capable of limiting myocardial infarct size. An additional aim was, with the use of microdialysis,[11,14] to quantify the myocardial norepinephrine concentrations during

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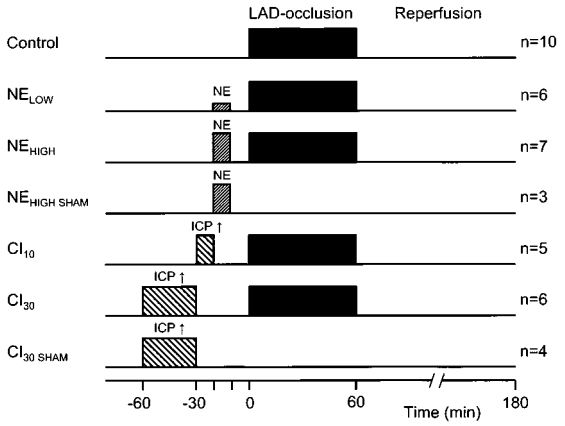

Figure 1. Seven experimental groups. Norepinephrine (NE) was infused at a rate of either 0.03 (NE_{low}) or 0.12 nmol/kg · min^{-1} (NE_{high} and $NE_{high\ sham}$) into the LAD. Global cerebral ischemia (CI), produced by elevating intracranial pressure (ICP), was maintained for either 10 minutes (CI_{10}) or 30 minutes (CI_{30} and $CI_{30\ sham}$). Infarct size was determined at the end of 120 minutes of reperfusion in all groups except in $NE_{high\ sham}$.

cerebral ischemia and exogenous norepinephrine infusions and to determine whether limitation of infarct size is mediated by attenuation of myocardial interstitial norepinephrine levels during the infarct-producing coronary artery occlusion.[15]

Materials and Methods

The present experiments were performed to conform with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and under the regulations of Erasmus University Rotterdam.

Experimental Groups

Forty-one crossbred Landrace×Yorkshire pigs of either sex (weight, 34±1 kg) were assigned to 1 of 7 experimental groups, of which in 6 groups myocardial infarct size was determined at the end of the protocol (Figure 1). Ten animals (control) underwent a 60-minute left anterior descending coronary artery (LAD) occlusion followed by 120-minute reperfusion, while in 13 animals the 60-minute LAD occlusion/reperfusion was preceded by a 10-minute norepinephrine infusion into the LAD at a rate of either 0.03 nmol/kg · min^{-1} (NE_{low}, n=6) or 0.12 nmol/kg · min^{-1} (NE_{high}, n=7). In 3 animals, the effects of the 10-minute high-dose norepinephrine infusion ($NE_{high\ sham}$) on myocardial function and metabolism were evaluated to assess whether this dose produced myocardial ischemia and asynchrony of contraction. Infarct size was not determined in these animals. In 5 animals a 10-minute period of global cerebral ischemia (CI_{10}) preceded the 60-minute LAD occlusion by 20 minutes, while in 6 animals the LAD occlusion was preceded by a 30-minute period of cerebral ischemia (CI_{30}) and 30 minutes of reperfusion. Finally, in 4 animals we studied whether 30 minutes of global cerebral ischemia per se ($CI_{30\ sham}$) damaged normal myocardium. Cerebral ischemia was achieved by infusion of artificial cerebrospinal fluid,[16] such that intracranial pressure increased to approximately 250 mm Hg (invariably above the systolic arterial pressure). In all groups, a 120-minute stabilization period followed the surgical procedures, after which baseline measurements were made. Microdialysis was performed in control, NE_{low}, NE_{high}, and CI_{30} groups. Dialysate samples were collected over 10-minute periods for determination of myocardial interstitial norepinephrine concentrations starting 90 minutes into the stabilization period, when norepinephrine concentrations had reached stable levels.[14,15] During the subsequent 30 minutes, baseline dialysate samples were collected. Plasma samples were obtained halfway through each 10-minute dialysate collection period. Animals encountering ventricular fibrillation during the protocol were allowed to complete the experiment when sinus rhythm could be restored by direct current countershock within 2 minutes.

Surgery

Overnight fasted pigs were sedated with ketamine (20 to 25 mg/kg IM; Apharmo), anesthetized with sodium pentobarbital (20 mg/kg IV; Apharmo), and intubated for ventilation with 30% oxygenated room air, while arterial blood gases were kept within the normal range. Catheters were inserted into the superior caval vein for infusion of sodium pentobarbital (10 to 15 mg/kg · h^{-1}) and saline. A fluid-filled catheter was placed in the descending aorta for measurement of aortic blood pressure and collection of blood samples, while a micromanometer-tipped catheter was inserted in the carotid artery and advanced into the left ventricle for measurement of left ventricular pressure (LVP) and its first derivative (LVdP/dt). After administration of pancuronium bromide (4 mg; Organon Teknika BV) and a midsternal thoracotomy, the heart was suspended in a pericardial cradle. An electromagnetic flow probe (Skalar) was placed around the ascending aorta for measurement of cardiac output, while the segment of the LAD between the first and the second diagonal branch was dissected free for placement of a Doppler flow probe (Triton Technology Inc) and a microvascular clamp. In NE_{low}, NE_{high}, and $NE_{high\ sham}$ groups, a small cannula was inserted into the LAD distal to the flow probe.

One microdialysis probe was implanted in the LAD area and one in the left circumflex coronary artery (LCx) area. In CI_{30}, a third probe was placed in the cortex of the brain. Perfusion of the probes started immediately after insertion.[14,15]

In $CI_{30\ sham}$ and $NE_{high\ sham}$ groups, pairs of ultrasound crystals were implanted in the midmyocardial layer of the LAD and LCx areas to assess regional myocardial wall function (Triton Technology Inc), while the great cardiac vein accompanying the LAD was cannulated for collection of blood samples. Finally, in the $NE_{high\ sham}$ group, the left atrium was also cannulated for injection of radioactive microspheres (^{113}Sn or ^{141}Ce, 15±1 [SD] μm) to determine the effect of norepinephrine on the distribution of myocardial blood flow.[1]

Two catheters were inserted into the left and right cerebral lateral ventricles through bore holes to produce cerebral ischemia.[17] A fluid-filled catheter was used for infusion of the artificial cerebrospinal fluid to elevate intracranial pressure, which was monitored with a micromanometer-tipped catheter.

Microdialysis

The polycarbonate dialysis membrane of the microdialysis probes (CMA/20, Carnegie Medicine) has a cutoff value of 20 kDa, a length of 10 mm, and a diameter of 0.5 mm. Cardiac probes were perfused with an isotonic Ringer's solution, and the cerebral probe was perfused with the artificial cerebrospinal fluid at a rate of 2 μL/min with the use of 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-norepinephrine as internal standard in 0.08N acetic acid. Plasma samples were drawn into chilled heparinized tubes containing 12 mg glutathione. All samples were stored at −80°C until analysis within the next 5 days.[14,15] In vivo probe recovery of norepinephrine, determined by retrodialysis and by direct comparison of hemomicrodialysis and plasma samples, is 52±1%.[14,15,18]

Infarct Size

At the end of the 120-minute reperfusion period, the area at risk was determined by intra-atrial infusion of 20 mL of 5% (wt/wt) fluorescein sodium.[19] After the heart was excised, the left ventricle was isolated and cut parallel to the atrioventricular groove into 5 slices of equal thickness. After the area at risk of each slice was demarcated on an acetate sheet under ultraviolet light, the slices were incubated in 0.125 g para-nitroblue tetrazolium (Sigma Chemical Co) per liter of phosphate buffer (pH 7.4) at 37°C for 30 minutes, and the nonstained pale infarcted area was also traced onto the sheet.

Myocardial infarct size was defined as the ratio of the summed infarct areas and summed areas at risk.[19]

Regional Myocardial Function and Perfusion

Percent systolic shortening (SS) was calculated as the difference in segment length at end diastole and the minimal segment length during systole divided by the segment length at end diastole. Asynchrony during norepinephrine infusion was assessed by determining the time interval between the occurrence of minimal segment length (L_{min}) in the LAD and LCx areas.

Myocardial O_2 extraction (%) was calculated as the ratio of the arterio–coronary venous O_2 content difference and the arterial O_2 content. At the end of the experiment, the heart was excised, and the LAD and LCx areas were separated and divided into 3 layers of equal thickness to determine the subendocardial (inner layer) and subepicardial (outer layer) blood flows and their ratios, with the use of standard techniques.[1]

Statistical Analysis

All data have been expressed as mean±SEM. Statistical significance ($P<0.05$) for changes in hemodynamics and norepinephrine concentrations was determined by 2-way ANOVA and 1-way ANOVA for repeated measures, followed by Dunnett's multiple comparison test. Statistical significance ($P<0.05$) for differences in infarct size was determined by 1-way ANOVA followed by Student's *t* test.

Results

Hemodynamics

Norepinephrine Infusions

Intracoronary norepinephrine infusion in the NE_{low} group produced an increase in maximal rise in LVP ($LVdP/dt_{max}$), reflecting an increase in regional contractility as the other cardiovascular variables remained unaffected (Table 1). During the 10-minute washout period, $LVdP/dt_{max}$ returned to baseline. During norepinephrine infusion in NE_{high} and $NE_{high\ sham}$ groups, mean arterial pressure decreased rapidly from 91±2 to 74±6 mm Hg, followed by a gradual recovery (Table 1). The decrease in cardiac output was responsible for the hypotension as systemic vascular resistance remained unchanged. Cardiac output decreased because the increase in heart rate was insufficient to compensate for the decrease in stroke volume. The latter occurred despite the increase in $LVdP/dt_{max}$ and was likely due to asynchrony of contraction (see below). All parameters recovered during the 10-minute washout period that preceded the 60-minute LAD occlusion.

During norepinephrine infusion in the $NE_{high\ sham}$ group, SS in the LAD area increased from 27±2% at baseline to 34±4%, while SS in the LCx area decreased from 18±1% to 13±1% (both $P<0.05$). These changes were accompanied by asynchrony of contraction between the LAD and LCx areas. Thus, whereas under baseline conditions L_{min} of both areas occurred at the end of global left ventricular systole, during norepinephrine infusion the occurrence of L_{min} in the LAD area preceded L_{min} in the LCx area by 119±2 ms ($P<0.05$). The latter was due to L_{min} in the LAD area occurring 56±15 ms before and L_{min} in the LCx area occurring 63±7 ms after closure of the aortic valves (both $P<0.05$ versus their respective baseline values). During washout, all wall function parameters returned to baseline values.

In the LAD area of $NE_{high\ sham}$, O_2 extraction decreased from 64±7% at baseline to 54±7% during norepinephrine infusion, indicating that O_2 delivery increased slightly in excess of the increase in myocardial O_2 demand. In addition, the arterio–coronary venous pH difference remained unchanged (0.06±0.01 at baseline and at the end of infusion). Moreover, the subendocardial to subepicardial blood flow ratio remained unchanged in both the LAD area (1.14±0.25 at baseline and 1.30±0.17 at the end of infusion) and the LCx area (1.21±0.07 and 1.24±0.05, respectively). Finally, SS in the LAD and LCx areas returned to baseline values immediately during the recovery period (24±3% and 17±1%, respectively), indicating that the norepinephrine infusion did not produce myocardial ischemia and stunning.

Cerebral Ischemia

Increasing intracranial pressure (12±2 mm Hg at baseline) to 250 mm Hg produced an immediate increase in mean aortic pressure in CI_{10}, CI_{30}, and $CI_{30\ sham}$ groups, which was initially the consequence of increases in both cardiac output and systemic vascular resistance (Table 1). However, after 5 minutes the tachycardia-mediated increase in cardiac output was exclusively responsible for the hypertension. Despite the increase in afterload, stroke volume was maintained, most likely because of enhanced myocardial contractility as $LVdP/dt_{max}$ increased up to 4 times its baseline value. The increase in coronary blood flow paralleled the increase in myocardial O_2 demand, reflected by the 150% increase in double product (heart rate×systolic arterial pressure).

Similar to earlier observations in dogs[20] and pigs,[11] the transient hyperdynamic phase was followed by a fall in mean arterial pressure below baseline levels at 10 minutes of cerebral ischemia, which was the result of systemic vasodilatation. Except heart rate, which remained slightly elevated, all other variables had recovered at 10 minutes. In CI_{30} and $CI_{30\ sham}$ groups, mean arterial pressure, cardiac output, and systemic vascular resistance did not change further during the remainder of the 30-minute period of cerebral ischemia, while heart rate returned to baseline levels and stroke volume increased. Except for mean arterial pressure and systemic vascular resistance, all other hemodynamic variables and intracranial pressure returned to baseline values during recovery (Table 1).

The increase in intracranial pressure decreased myocardial O_2 extraction from 64±5% at baseline to 58±6% at 5 minutes but did not change the arterio–coronary venous pH difference (0.04±0.01 at baseline and at 5 minutes), indicating the absence of myocardial ischemia. The elevation of intracranial pressure decreased SS from 24±1% to 16±2% at 2 minutes, but SS had already recovered to 23±1% at 5 minutes and to 26±1% at 10 minutes, with no evidence of depressed regional wall function during the remainder of the 30-minute period (27±1%) or the subsequent recovery phase (23±3%).

LAD Occlusion and Reperfusion

In the control group, mean arterial pressure decreased secondary to the decrease in cardiac output during the 60-minute LAD occlusion and did not change further during reperfusion (Table 2). Heart rate increased slightly, but insufficiently to compensate for the decrease in stroke volume.

TABLE 1. Cardiovascular Hemodynamics During Norepinephrine Infusion or Cerebral Ischemia

			Δ From Baseline				
	Treatment	Baseline	2 min	5 min	10 min	30 min	Recovery
Mean arterial pressure, mm Hg	NE_{low}	93±3	0±3	2±2	3±3	...	−1±2
	$NE_{high}+NE_{high\ sham}$	91±2	−15±6*	−17±5*	−8±4	...	−2±2
	CI_{10}	92±5	80±10*	53±13*	−11±5	...	−16±12
	$CI_{30}+CI_{30\ sham}$	90±4	83±6*	67±8*	−19±4*	−17±5*	−20±4*
Cardiac output, L · min^{-1}	NE_{low}	2.9±0.2	0.0±0.1	0.0±0.1	0.0±0.1	...	−0.1±0.1
	$NE_{high}+NE_{high\ sham}$	2.5±0.2	−0.4±0.2*	−0.5±0.2*	−0.2±0.1	...	−0.1±0.1
	CI_{10}	3.8±0.5	0.7±0.2*	2.1±0.4*	0.7±0.3	...	−0.3±0.6
	$CI_{30}+CI_{30\ sham}$	3.1±0.2	0.7±0.2*	2.2±0.3*	0.4±0.1*	0.9±0.2*	−0.2±0.4
Systemic vascular resistance,	NE_{low}	33±3	0.2±0.8	0.4±0.7	1.1±0.7	...	1.1±1.1
mm Hg/L · min^{-1}	$NE_{high}+NE_{high\ sham}$	37±3	−0.6±1.4	0.7±0.9	0.6±1.0	...	1.2±0.9
	CI_{10}	26±3	15.9±4.6*	1.4±4.4	−7.4±1.9*	...	−3.6±1.0*
	$CI_{30}+CI_{30\ sham}$	30±2	18.8±4.2*	1.8±3.2	−8.8±1.3*	−11.0±1.5*	−9.0±2.1*
Heart rate, bpm	NE_{low}	107±7	1±1	1±2	1±2	...	1±3
	$NE_{high}+NE_{high\ sham}$	117±6	13±4*	15±5*	12±5*	...	2±2
	CI_{10}	112±5	37±8*	68±12*	19±13	...	17±10
	$CI_{30}+CI_{30\ sham}$	101±3	46±5*	79±6*	22±4*	2±3	9±7
Stroke volume, mL	NE_{low}	27±4	1.1±1.5	1.5±1.4	1.2±1.2	...	0.5±1.1
	$NE_{high}+NE_{high\ sham}$	22±2	−5.4±1.9*	−6.4±1.6*	−3.8±1.7	...	−1.1±0.7
	CI_{10}	33±3	−2.6±2.2	0.2±3.8	1.4±3.0	...	−5.8±3.0
	$CI_{30}+CI_{30\ sham}$	30±2	−4.4±1.8*	−0.8±1.5	−1.8±1.2	8.0±1.6*	−4.2±3.2
$LVdP/dt_{max}$, mm Hg · s^{-1}	NE_{low}	1560±110	760±100*	790±90*	850±60*	...	−60±70
	$NE_{high}+NE_{high\ sham}$	2030±200	860±210*	890±160*	1270±120*	...	−150±70
	CI–10	1790±170	2810±660*	4650±910*	−310±410	...	410±560
	$CI_{30}+CI_{30\ sham}$	1640±120	2570±440*	5010±340*	160±230	−90±170	50±220
Left ventricular end-diastolic	NE_{low}	8±2	1.2±0.5	1.3±0.6	1.9±0.8	...	1.1±0.6
pressure, mm Hg	$NE_{high}+NE_{high\ sham}$	8±1	−1.7±0.8	−2.0±0.8*	−1.4±0.6	...	0.7±0.6
	CI_{10}	9±1	8.5±1.7*	1.5±1.2	0.2±2.4	...	−2.5±1.8
	$CI_{30}+CI_{30\ sham}$	7±1	10.6±2.5*	0.9±1.7	−1.2±1.2	1.8±1.5	0.0±0.9
Coronary blood flow, mL/min · g^{-1}	NE_{low}	1.7±0.2	0.3±0.2	0.3±0.2	0.3±0.1	...	0.0±0.2
	$NE_{high}+NE_{high\ sham}$	1.0±0.1	0.0±0.1	0.1±0.1	0.3±0.1	...	0.0±0.1
	CI_{10}	1.0±0.2	1.0±0.2*	0.7±0.1*	0.0±0.1	...	−0.1±0.1
	$CI_{30}+CI_{30\ sham}$	1.3±0.1	1.3±0.2*	1.4±0.3*	−0.1±0.1	0.2±0.2	−0.1±0.1

Data are mean±SEM; n=6 (NE_{low}), n=10 ($NE_{high}+NE_{high\ sham}$), n=5 ($CI_{10}$), n=10 ($CI_{30}+CI_{30\ sham}$).
*$P<0.05$ vs baseline.

Pretreatment with norepinephrine had no effect on the hemodynamic responses during the subsequent LAD occlusion and reperfusion in either NE_{low} or NE_{high}. In CI_{10} and CI_{30} groups, mean arterial pressure did not further decrease during the LAD occlusion, most likely because systemic vascular resistance, which was still below baseline levels at the onset of LAD occlusion, recovered.

Myocardial Infarct Size

The area at risk was identical in all experimental groups (Figure 2). Infarct size was 84±3% in control and 80±3% in NE_{low} groups but only 65±2% in the NE_{high} group ($P<0.05$). Cerebral ischemia had no effect on infarct size development during the 60-minute LAD occlusion, since in CI_{10} and CI_{30} groups infarct size was 83±4% and 82±3%, respectively. Cerebral ischemia per se did not cause irreversible damage, since in none of the $CI_{30\ sham}$ animals was infarct tissue detected.

Myocardial Interstitial Norepinephrine Concentrations

The myocardial interstitial norepinephrine levels in the LAD area increased from 0.8±0.2 to 2.2±0.5 nmol/L in the NE_{low} group and to 12.2±5.9 nmol/L in the NE_{high} group during norepinephrine infusion (both $P<0.05$; Figure 3). Despite the intracoronary route, there was some spillover in the NE_{high} group, as evidenced by small transient increments of norepinephrine in plasma from 0.2±0.1 to 1.0±0.2 nmol/L and in the interstitium of the LCx area from 1.1±0.3 to 2.4±1.2 nmol/L (both $P<0.05$; Figure 3).

In CI_{30}, cerebral interstitial norepinephrine levels increased from 0.9±0.4 nmol/L at baseline to 6.1±1.9 nmol/L at 10

TABLE 2. Cardiovascular Hemodynamics During Coronary Occlusion and Reperfusion

	Treatment	Preocclusion Values	End-Occlusion Values	End-Reperfusion Values
Mean arterial pressure, mm Hg	Control	90±2	75±2*	76±6*
	NE_{low}	93±4	72±6*	70±5*
	NE_{high}	87±4	77±5	77±4
	CI_{10}	75±9	74±5	65±5
	CI_{30}	65±4	61±5	66±4
Cardiac output, L · min^{-1}	Control	2.6±0.2	2.1±0.1*	2.1±0.2*
	NE_{low}	2.8±0.2	2.3±0.2*	1.9±0.1*
	NE_{high}	2.2±0.2	1.9±0.2*	1.8±0.2*
	CI_{10}	3.5±0.4	2.9±0.3	2.5±0.4
	CI_{30}	2.9±0.3	2.2±0.2*	2.0±0.1*
Systemic vascular resistance,	Control	38±3	37±3	38±4
mm Hg/L · min^{-1}	NE_{low}	34±4	32±3	38±4
	NE_{high}	41±4	43±4	45±5
	CI_{10}	22±3	27±4	28±4*
	CI_{30}	24±2	28±2*	33±2*
Heart rate, bpm	Control	115±4	123±7	139±8*
	NE_{low}	108±9	112±9	119±13
	NE_{high}	124±6	133±11	136±11
	CI_{10}	129±7	119±9	120±7
	CI_{30}	112±6	112±5	115±6
Stroke volume, mL	Control	22±2	18±1*	16±1*
	NE_{low}	27±4	22±3	17±2
	NE_{high}	18±2	14±2*	13±1*
	CI_{10}	28±4	25±4	21±3
	CI_{30}	25±2	20±2*	18±1*
$LVdP/dt_{max}$, mm Hg · s^{-1}	Control	1610±100	1480±100	1640±260
	NE_{low}	1500±110	1350±110	1250±100
	NE_{high}	1980±280	2060±350	2050±340
	CI_{10}	2200±410	1630±140	1350±140
	CI_{30}	1710±320	1250±160	1170±100
Left ventricular end-diastolic pressure,	Control	7±1	9±1	10±1*
mm Hg	NE_{low}	9±2	10±1	10±1
	NE_{high}	8±2	10±2	12±1
	CI_{10}	7±3	13±3	13±1
	CI_{30}	6±1	9±2*	12±1*

Data are mean±SEM; n=10 (control), n=6 (NE_{low}), n=7 (NE_{high}), n=5 (CI_{10}), n=6 (CI_{30}). Preocclusion values of the norepinephrine and cerebral ischemia groups correspond to the recovery values in Table 1.

*$P<0.05$ vs preocclusion values.

minutes of intracranial pressure elevation and up to 8.3±1.8 nmol/L at 30 minutes (not shown in Figure 3). On cerebral reperfusion, interstitial levels initially increased further to 12.3±2.3 nmol/L but returned to baseline during the remainder of the 30-minute recovery period. Cerebral ischemia resulted in a transient tripling of interstitial norepinephrine levels in both the LAD and LCx areas and in a 20-fold increase in plasma norepinephrine levels (Figure 3).

In control, NE_{low}, NE_{high}, and CI_{30} groups, norepinephrine levels increased progressively during LAD occlusion by up to approximately 500-fold and recovered during reperfusion, independent of the preceding intervention (Figure 4). There was no correlation ($r=0.03$) between the maximum interstitial norepinephrine levels during LAD occlusion and myocardial infarct size.

Discussion

The major findings of the present study are as follows: (1) global cerebral ischemia, produced by either a 10-minute or a 30-minute elevation of intracranial pressure, which by itself produced no irreversible myocardial damage, had no effect on myocardial infarct size produced by 60-minute coronary artery occlusion; (2) intracoronary infusion of 0.03 nmol/kg · min^{-1} norepinephrine produced increases in myocardial in-

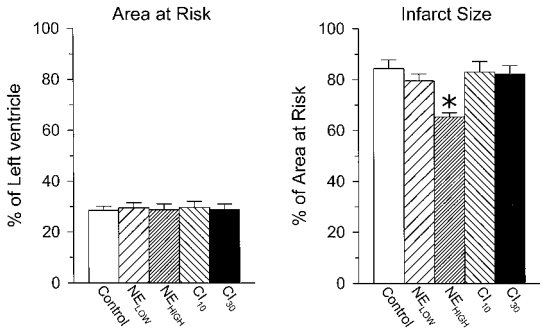

Figure 2. Area at risk and infarct size for the 5 experimental groups in which the LAD was occluded for 60 minutes and reperfused for 120 minutes. Global cerebral ischemia alone ($CI_{30\ sham}$) did not cause irreversible myocardial damage (not shown). *$P<0.05$ vs control. For further details see Figure 1.

terstitial levels similar to those produced by cerebral ischemia and also did not limit myocardial infarct size; (3) conversely, intracoronary infusion of 0.12 nmol/kg · min^{-1} of norepinephrine, which resulted in 5-fold higher myocardial interstitial norepinephrine levels than cerebral ischemia and low-dose norepinephrine, was capable of limiting myocardial infarct size; (4) the cardioprotection by exogenous norepinephrine was not caused by ischemic preconditioning; and (5) this protection was not associated with a blunting of the progressive increase in myocardial interstitial norepinephrine levels during coronary artery occlusion.

Catecholamines and Myocardial Injury

The relation between catecholamines and myocardial injury was first established by Rona and coworkers,[21,22] who showed some 40 years ago that administration of high systemic doses of isoproterenol produced focal necrotic lesions in normal rat hearts.

Elevation of intracranial pressure is well recognized as a cause of myocardial dysfunction and injury. Brain death caused by increased intracranial pressure produces echocardiographic alterations, hemodynamic instability, and contraction band necrosis, all of which have been suggested to be the result of massive neuronal depolarization and release of catecholamines.[23–25] These clinical observations initiated a large number of experimental investigations in which deleterious effects of brain death on function and integrity of normal myocardium were found, but generally no or only minimal focal myocardial necrosis could be demonstrated.[26,27]

In view of the massive myocardial norepinephrine release during coronary artery occlusion,[15,28] it could be hypothesized that catecholamines may contribute to the development of irreversible injury during a coronary artery occlusion. Several,[29,30] although certainly not all,[31,32] studies have reported that β-adrenoceptor blockade slows the development of myocardial infarction. In contrast, depletion of cardiac norepinephrine stores by reserpination did not limit myocardial infarct size in rabbits[9] and dogs,[10] suggesting that endogenous catecholamines do not contribute to irreversible damage.

In contrast to the potentially deleterious effects of norepinephrine on normal and ischemic myocardium, this catecholamine has also been implicated in mediating cardioprotection by ischemic preconditioning. Thus, Toombs et al[9] showed that in rabbits the protection by ischemic preconditioning was abolished when catecholamine stores in sympathetic nerve endings were depleted by reserpine. Furthermore, Thornton et al[12] demonstrated in the same species that tyramine-induced norepinephrine release 10 minutes before a 30-minute coronary artery occlusion also protected the myocardium. This cardioprotective action of catecholamines has been confirmed in other species such as the rat[13] and the dog.[10] We now show that a high dose of norepinephrine can also protect the porcine myocardium.

Our data on wall function, myocardial blood flow, O_2 extraction, and proton release indicate that the high dose of norepinephrine did not produce myocardial ischemia and therefore did not protect the myocardium by ischemic preconditioning. The degree of protection afforded by norepinephrine is less than reported for ischemic preconditioning but similar to that produced by other nonischemic stimuli, such as ventricular pacing[33] and pharmacological agents such as the K^+_{ATP} channel openers.[34] Since all these stimuli have in common that they ultimately activate K^+_{ATP} channels, it is tempting to speculate that norepinephrine also protected via α_1-adrenoceptor–mediated protein kinase C activation and subsequent opening of (mitochondrial) K^+_{ATP} channels.[35] Another mechanism by which norepinephrine might protect the myocardium is via a blunted release in catecholamines during the sustained ischemic episode.[36] However, pretreatment with norepinephrine did not modify the release of cardiac norepinephrine during sustained myocardial ischemia in the present study, implying that the norepinephrine-mediated cardioprotection is not related to a blunting of the ischemia-induced increase in norepinephrine levels.

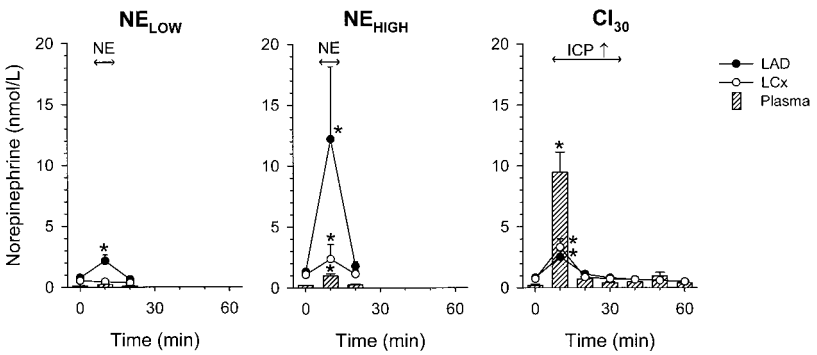

Figure 3. Norepinephrine levels in plasma and in the interstitium of the LAD area and the LCx area during the 10-minute norepinephrine infusions and during 30 minutes of cerebral ischemia. *$P<0.05$ vs baseline. For further details see Figure 1.

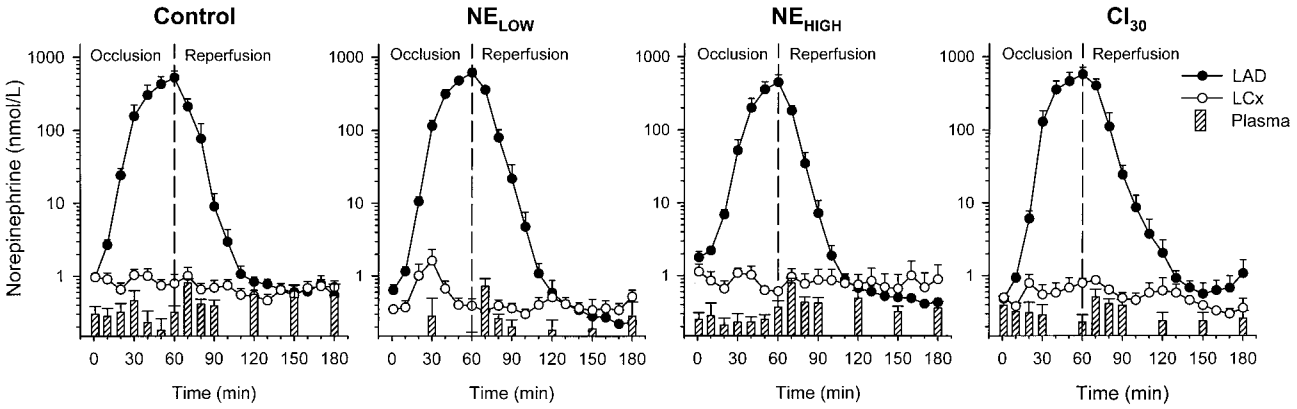

Figure 4. Norepinephrine levels in plasma, the LAD area, and the LCx area during 60-minute LAD occlusion and 120-minute reperfusion. Notice that in the LAD area levels increased 500-fold during LAD occlusion independent of the preceding intervention. Levels in the LCx area and plasma remained unchanged.

Finally, the present study clarifies another issue on the role of norepinephrine in cardioprotection. Przyklenk et al[6] demonstrated that myocardial ischemia also elicited cardioprotection in adjacent virgin myocardium and speculated that this might have been triggered by a substantial catecholamine release in that adjacent region. However, we now show that norepinephrine levels in the normal (LCx-perfused) myocardium remained unaltered during and after the 60-minute LAD occlusion (Figure 4), even though the interstitial norepinephrine levels in the LAD area were 100-fold higher than the value observed after 10 minutes of ischemia, corresponding to the period used by Przyklenk et al[6] to precondition the adjacent virgin myocardium.

Cerebral Ischemia as a Stimulus for Cardioprotection

Transient ischemia in small intestines, kidneys, and skeletal muscle before a coronary artery occlusion can also be cardioprotective.[7,8] We therefore hypothesized that cerebral ischemia might similarly protect the myocardium, especially because cerebral ischemia is associated with substantial norepinephrine release, one of the mediators involved in cardioprotection by ischemic preconditioning. However, transient cerebral ischemia did not reduce myocardial infarct size in the present study. The explanation for the lack of protection might be 2-fold. First, 30 minutes of cerebral ischemia did not produce myocardial ischemia and could therefore not protect the myocardium via ischemic preconditioning. Second, although myocardial interstitial norepinephrine levels increased during cerebral ischemia, the rise was much less than during infusion of the high dose of norepinephrine, which elicited cardioprotection. This is further corroborated by our findings with the low dose of norepinephrine, which produced interstitial myocardial norepinephrine levels similar to those produced by cerebral ischemia and was also ineffective in protecting the heart.

It could be argued that even the 30-minute global cerebral ischemia (CI_{30}) was too short to elicit cardioprotection. However, there is ample evidence that the intensity of the preconditioning stimulus is more important than its duration.[37] Moreover, because the elevation of myocardial interstitial norepinephrine levels occurred exclusively during the first 10 minutes of cerebral ischemia, it is unlikely that extending the period of cerebral ischemia would produce cardioprotection. On the contrary, it might be argued that the duration of the intracranial pressure elevation and recovery phase lasted too long since the maximum myocardial interstitial norepinephrine levels reached their peak during the first 10 minutes, so that a potential effect of that stimulus was lost by the time (50 minutes later) the LAD was occluded. This is supported by observations that the memory for cardioprotection is shorter when stimuli are used that do not cause myocardial ischemia.[33,34] However, when cerebral ischemia was maintained for only 10 minutes and cerebral reperfusion was shortened to 20 minutes (CI_{10}), infarct size after the 60-minute coronary artery occlusion was also not different from control.

Finally, it can be excluded that a protective effect of transient global cerebral ischemia during LAD occlusion was masked by irreversible myocardial damage produced by transient global cerebral ischemia before LAD occlusion, since irreversible damage could not be detected in the animals subjected to only 30 minutes of cerebral ischemia ($CI_{30\ sham}$). This observation is in agreement with most experimental studies that have generally reported minimal or no focal myocardial necrosis after cerebral ischemia.[26,27]

Conclusions

In conclusion, global cerebral ischemia preceding a coronary artery occlusion did not modify myocardial infarct size, which is likely related to the modest increase in myocardial norepinephrine levels during cerebral ischemia. The infarct size limitation by the high dose of norepinephrine was not associated with a blunting of the increase in myocardial interstitial norepinephrine levels during coronary occlusion.

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