Catecholamine handling in the porcine heart: a microdialysis approach

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Lameris, T. W., A. H. van den Meiracker, F. Boomsma, G. Alberts, S. de Zeeuw, D. J. Duncker, P. D. Verdouw, and A. J. Man in 't Veld. Catecholamine handling in the porcine heart: a microdialysis approach. *Am. J. Physiol.* 277 (*Heart Circ. Physiol.* 46): H1562–H1569, 1999.—Experimental findings suggest a pronounced concentration gradient of norepinephrine (NE) between the intravascular and interstitial compartments of the heart, compatible with an active neuronal reuptake (U1) and/or an endothelial barrier. Using the microdialysis technique in eight anesthetized pigs, we investigated this NE gradient, both under baseline conditions and during increments in either systemic or myocardial interstitial fluid (MIF) NE concentration. At steady state, baseline MIF NE (0.9 ± 0.1 nmol/l) was higher than arterial NE (0.3 ± 0.1 nmol/l) but was not different from coronary venous NE (1.5 ± 0.3 nmol/l). Local U1 inhibition raised MIF NE concentration to 6.5 ± 0.9 nmol/l. During intravenous NE infusions (0.6 and 1.8 nmol·kg^{-1}·min^{-1}), the fractional removal of NE by the myocardium was 79 ± 4% to 69 ± 3%, depending on the infusion rate. Despite this extensive removal, the quotient of changes in MIF and arterial concentration (ΔMIF/ΔA ratio) for NE were only 0.10 ± 0.02 for the lower infusion rate and 0.11 ± 0.01 for the higher infusion rate, whereas U1 blockade caused the ΔMIF/ΔA ratio to rise to 0.21 ± 0.03 and 0.36 ± 0.05, respectively. From the differences in ΔMIF/ΔA ratios with and without U1 inhibition, we calculated that 67 ± 5% of MIF NE is removed by U1. Intracoronary infusion of tyramine (154 nmol·kg^{-1}·min^{-1}) caused a 15-fold increase in MIF NE concentration. This pronounced increase was paralleled by a comparable increase of NE in the coronary vein. We conclude that U1 and extraneuronal uptake, and not an endothelial barrier, are the principal mechanisms underlying the concentration gradient of NE between the interstitial and intravascular compartments in the porcine heart.

norepinephrine; spillover; pig; myocardial interstitium

SEVERAL EXPERIMENTAL FINDINGS suggest that in the heart a pronounced concentration gradient for norepinephrine (NE) exists between the interstitial and intravascular compartments. Silverberg et al. (31) showed that the coronary sinus NE concentration induced by an NE infusion that led to only a small increase in the heart rate was eight times higher than that induced by stellate ganglion stimulation that caused a similar increase in heart rate. From tracer dilution experiments Cousineau et al. (8) estimated a ratio of 15% between the interstitial and arterial compartments in canine hearts. More recently, Obst et al. (29), using ultrafiltration of interstitial fluid from isolated perfused rat hearts, found ratios of interstitial transudate to arterial concentrations of NE of 0.14 to 0.77, depending on the concentration of NE administered. These investigators interpreted these findings as suggesting that the neuronal uptake of NE is the most important determinant for maintaining a concentration gradient between the circulatory and interstitial compartments. Because of the dense sympathetic innervation, especially in the heart, neuronal reuptake (U1) of NE could be an important determinant for maintaining such a gradient (17, 19). However, other investigators have suggested that the concentration gradient is caused by a physical barrier of the blood vessel wall as well (7, 8, 25, 29, 30, 33).

A profound understanding of catecholamine kinetics is invaluable when interpreting catecholamine data and their representation of sympathetic activity. Using the isotope dilution method and arteriovenous sampling, Esler et al. (16) introduced regional spillover as a kinetic parameter that aims to reflect the rate of NE entering the circulation rather than true production. In a recent study, Kopin et al. (22) have modified this technique and introduced a new method to estimate neuronal release that is based on the measurements of the specific activities of radiolabeled NE and its extraneuronal metabolite, normetanephrine, in plasma. Neuronal release of NE into the interstitial space is estimated as the sum of the spillover of NE from the interstitium to the circulation and the uptake of released NE from the interstitium. Nonetheless, the interstitial compartment can only be monitored either by estimation through the application of mathematical kinetic modeling or with the use of in vitro or semi-in vivo preparations. The microdialysis technique, however, allows for the accurate estimation of the concentration of catecholamines in the myocardial interstitial fluid (MIF) in vivo (15, 18, 32, 37). The technique should allow for measurement of the hitherto unquantifiable amount of NE that is released but taken up before reaching the vascular compartment, thus filling the gaps in existing kinetic models.

In a series of experiments in which either the circulatory or the interstitial NE concentration was increased, we investigated how the concentration of NE in the MIF is related to its concentration in the arterial and coronary venous circulation. The importance of U1 and extraneuronal uptake to this relationship was explored by adding desipramine [desmethylimipramine (DMI)], a well-known U1 inhibitor, to the dialysate of one of the microdialysis probes and performing experiments with

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isoproterenol (Iso), a catecholamine that is not handled by U1. Furthermore, NE concentrations in MIF, arterial plasma, and the coronary effluent at baseline and during infusion of NE provided an estimate of spillover, uptake, and release of NE.

METHODS

Animal care. All experiments were performed in accordance with both the "Guiding Principles for Research Involving Animals and Human Beings" as approved by the Council of the American Physiological Society and the regulations of the Animal Care Committee of the Erasmus University of Rotterdam (The Netherlands).

Surgical procedure. After an overnight fast, crossbred Landrace × Yorkshire pigs of either sex (30–35 kg, $n = 8$) were sedated with ketamine (Ketalin; 20–25 mg/kg im) and anesthetized with pentobarbital sodium (Narcovet; 20 mg/kg iv). The animals were 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 ranges: pH between 7.35 and 7.45, P_{CO_2} between 35 and 45 mmHg, and P_{O_2} between 100 and 150 mmHg.

Catheters were positioned in the superior caval vein for continuous administration of pentobarbital sodium (10–15 $mg \cdot kg^{-1} \cdot h^{-1}$) and Haemaccel for replacement of blood withdrawn during sampling. In the descending aorta, a fluid-filled catheter was placed for monitoring of aortic blood pressure and for withdrawal of blood samples. Through the left carotid artery a micromanometer-tipped catheter (B. Braun Medical, Uden, The Netherlands) was inserted into the left ventricle for measurement of left ventricular pressure and, by electrical differentiation, the maximum of its first derivative (LV dP/dt_{max}). After pancuronium bromide (Pavulon; 4 mg) was administered, a midsternal thoracotomy was performed, and the heart was suspended in a pericardial cradle. An electromagnetic flow probe (Skalar, Delft, The Netherlands) was then placed around the ascending aorta for measurement of aortic blood flow (cardiac output). A proximal segment of the left anterior descending coronary artery (LAD) was dissected free for placement of a Doppler flow probe. Distal to this site, a small cannula (1.3-mm outer diameter) was inserted into the LAD for the administration of tyramine.

The microdialysis catheters were implanted in the tissue with the help of a steel guiding needle and split plastic tubing. Three microdialysis probes were inserted into the left ventricular myocardium: one in the region of the left circumflex coronary artery (LCX) and two in the area perfused by the LAD. To achieve local U1 inhibition, one of the LAD probes was coperfused with DMI (100 μM) (36). In addition, microdialysis probes were placed in the right carotid artery and the anterior interventricular coronary vein that drains the territory perfused by the LAD.

Dialysis methodology. For microdialysis, CMA/20 probes (Carnegie Medicine, Stockholm, Sweden) were used. The polycarbonate dialysis membrane of these probes has a cutoff value of 20 kDa, a length of 10 mm, and a diameter of 0.5 mm. The probes were perfused with an isotonic Ringer solution at a rate of 2 μl/min using a CMA/100 microinjection pump. Dialysate volumes of 20 μl (sampling time 10 min) were collected in microvials containing 20 μl of a solution of 2% (wt/vol) EDTA and 150 nM Epinine as internal standard in 0.08 N acetic acid. Sampling was started immediately after the catheters were inserted. The plasma samples were drawn into chilled heparinized tubes containing 12 mg of glutathione, and, like the microdialysis samples, stored at −80°C and analyzed within the next 5 days (5).

Determination of in vivo probe recovery. Probe recovery is defined as the quotient of dialysate catecholamine concentration and the actual catecholamine concentration in the medium that is dialyzed. In vivo probe recovery was determined separately for blood as well as myocardial intercellular fluid. Probe recovery for blood was estimated by comparing catecholamine concentrations in plasma with catecholamine concentrations in the corresponding dialysate of the microdialysis probe in the carotid artery (Fig. 1).

Recovery of NE for the probes in the myocardium was estimated by applying the retrodialysis method. This method relies on the assumptions that diffusion of a substance into the microdialysis probe equals the outward diffusion and that the diffusion characteristics of the calibrator match those of the analyte (Fig. 2) (24, 35). Furthermore, this method will only provide an estimation of the concentration of the analyte at the outer membrane of the microdialysis probe, rather

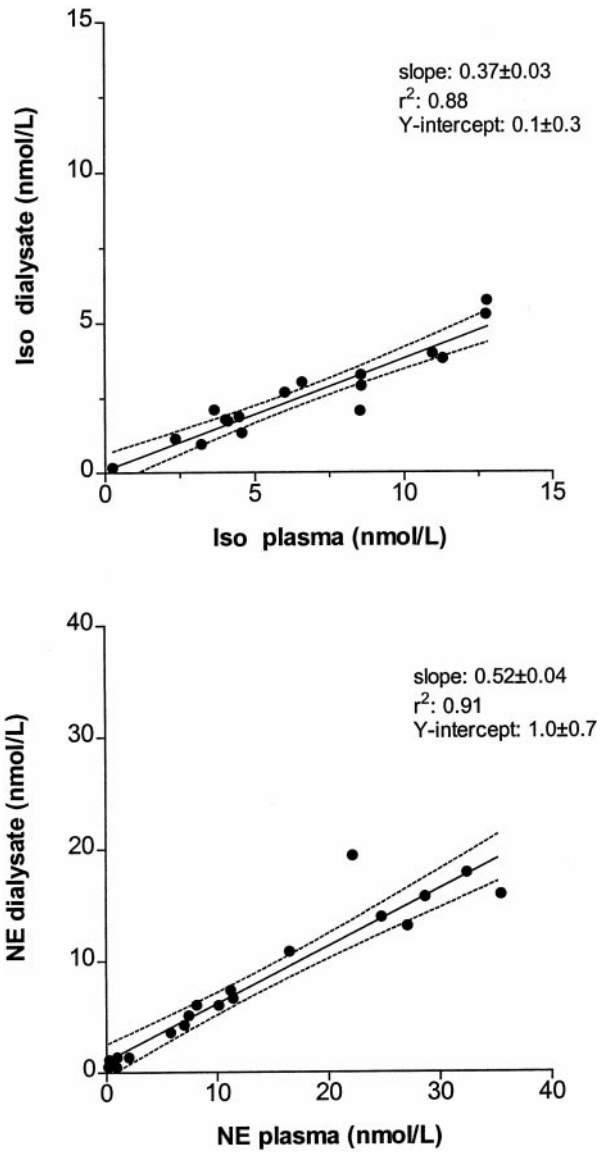

Fig. 1. Determination of in vivo recovery for isoproterenol (Iso; *top*) and norepinephrine (NE; *bottom*): arterial microdialysis vs. arterial sampling. Dotted lines indicate 95% confidence limits. Data are presented as 17 data points for 7 animals.

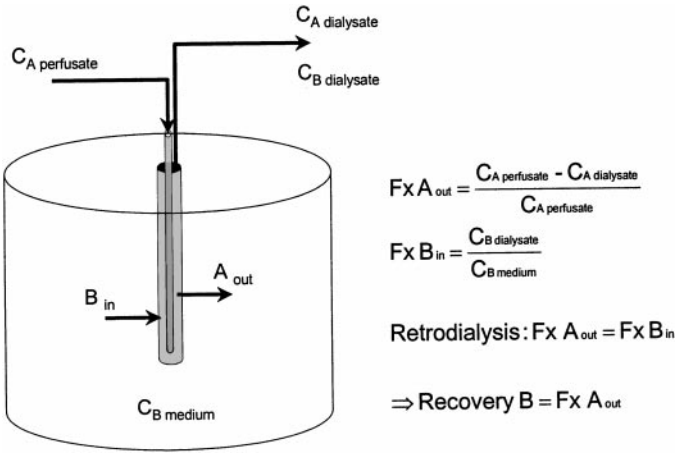

Fig. 2. Diagram of retrodialysis method for determination of in vivo recovery. C_A, concentration of *calibrator A*; C_B, concentration of *analyte B*; FxA_{out}, fraction of *calibrator A* that has diffused out of perfusate into medium; FxB_{in}, fraction of *analyte B* that has diffused out of medium into dialysate, i.e., recovery of *analyte B*.

than the mean interstitial concentration. Ideally, although the calibrator should not have any pharmacodynamic effects, both substances should have the same pharmacokinetic properties. Consequently, the concentration of the calibrator in the perfusate should be as low as possible to prevent any significant influence on the metabolism of the analyte, e.g., saturation of active uptake mechanisms such as U1.

In this study *l*-erythro-α-methylnorepinephrine (AMN) was chosen as the calibrator for probe recovery for NE. This AMN isomer is considered to be a "false transmitter"; it is handled like NE, although it does not share its pharmacodynamic effects. Retrodialysis was performed in four animals for the length of the whole experiment. The average percentage of loss of AMN, i.e., in vivo probe recovery for NE in MIF, was based on the data derived from the LAD and LCX probes of all four animals. This method revealed a probe recovery of 52 ± 1% (2 probes in 4 animals). Comparison of the concentration of NE in arterial plasma with the NE concentration in the dialysate obtained from the probe positioned in the carotid artery showed a similar value for probe recovery of NE (52 ± 4%, Fig. 1). For Iso, using a similar approach, the in vivo probe recovery was 37 ± 3% (Fig. 1).

Protocol. After 120–150 min, steady-state conditions were reached at baseline. Thereafter, NE and Iso were intravenously infused consecutively for 30 min for each dose, followed by a 30-min intracoronary infusion of tyramine. After each infusion, a 30-min stabilization period was introduced, allowing for a complete washout of the infused substances and return to baseline conditions. The infusion rates of Iso (0.16 and 0.48 $nmol \cdot kg^{-1} \cdot min^{-1}$) and tyramine (154 $nmol \cdot kg^{-1} \cdot min^{-1}$) were chosen to correspond with the hemodynamic response of the NE infusions (0.6 and 1.8 $nmol \cdot kg^{-1} \cdot min^{-1}$) (21). At the end of the experiments, the pigs were killed with an overdose of pentobarbital.

Analytic procedure. Plasma catecholamines were determined by HPLC with fluorimetric detection after liquid-liquid extraction and derivatization with the fluorogenic agent *N*,*N*′-diphenylethylenediamine (DPE) (34). For microdialysis samples, the catecholamines were not extracted before fluorimetric detection with HPLC but were directly derivatized according to the procedure described by Alberts et al. (3). This method suppresses the interference of in vivo factors on derivatization, thereby improving sensitivity.

Reagents and pharmaceuticals. Ketalin and Narcovet were obtained from Apharmo (Arnhem, The Netherlands), Pavulon from Organon Teknica (Boxtel, The Netherlands), Ringer solution from Baxter (Uden, The Netherlands), Haemaccel from Behringwerke (Marburg, Germany), and Epinine from Zambon (Milan, Italy). Tyramine, NE, and Iso for infusions were obtained from the Department of Pharmacy (University Hospital, Rotterdam, The Netherlands). DMI, bicine, NE, and AMN were purchased from Sigma (St. Louis, MO); EDTA, *N*-ethylmaleimide (NEM), and hydrochloric acid were from Merck (Darmstadt, Germany); potassium ferricyanide was from Aldrich (Bornem, Belgium); L-glutathione was from Fluka (Buchs, Switzerland); and acetic acid and acetonitrile were from Baker (Deventer, The Netherlands). DPE was prepared as reported previously (34).

Statistics and calculations. Results are expressed as means ± SE. NE and Iso concentrations obtained with microdialysis were corrected for probe recovery. Baseline values were determined by averaging the data from the steady state before the infusions. During the infusions of NE and Iso, the cardiac extraction (E) was calculated as

$$E\ (\%) = \frac{\Delta A - \Delta V}{\Delta A} \cdot 100 \qquad (1)$$

where ΔA and ΔV are the changes from baseline of arterial and coronary venous concentrations, respectively. The ratio of interstitial NE to arterial plasma NE is presented as

$$\Delta MIF/\Delta A\ ratio = \frac{\Delta MIF}{\Delta A} \qquad (2)$$

where ΔMIF is the change from baseline of the myocardial interstitial fluid concentration.

The percentage of NE that can be recovered from the MIF and taken up by U1 (FxU1) can be calculated from the difference between the ΔMIF/ΔA ratio with and without U1 inhibition as

$$Fx\ U1\ (\%) = \frac{\Delta MIF/\Delta A_{DMI} - \Delta MIF/\Delta A}{\Delta MIF/\Delta A_{DMI}} \cdot 100 \qquad (3)$$

where $\Delta MIF/\Delta A_{DMI}$ is the ΔMIF/ΔA ratio with U1 inhibition. The percentage of MIF NE that is originating from the circulation (MIF NE_A) at baseline can be estimated as follows

$$MIF\ NE_A\ (\%) = \frac{\Delta MIF/\Delta A\ ratio \cdot NE_A}{NE_{MIF}} \cdot 100 \qquad (4)$$

where NE_A and NE_{MIF} are the baseline NE concentrations in arterial plasma and MIF, respectively.

Although no radiolabeled material was used in the present study, the method introduced by Kopin and colleagues (22) can still be applied to estimate neuronal release by replacing the specific activity of radiolabeled NE with the ratio of changes from baseline of the NE concentration in arterial and coronary venous plasma (R_A and R_V, respectively) and MIF (R_{MIF}) during systemic infusion of NE. The use of this ratio is based on the assumptions that endogenous NE is reflected by the baseline NE concentration and that the change in its release is negligible compared with the change in NE concentrations induced by infused NE. According to these revised equations, spillover (SO) can be calculated as

$$SO = Q \cdot NE_A \cdot (1 - E) \cdot \left[\left(\frac{R_A}{R_V}\right) - 1\right] \qquad (5)$$

where Q is the coronary plasma flow (CPF) that is calculated from the coronary blood flow and the hematocrit, which was estimated using the plasma hemoglobin concentration. The uptake of released NE (Ur) from the interstitium can be estimated by using R_{MIF} in an analogous equation

$$Ur = Q \cdot NE_A \cdot E \cdot \left[\left(\frac{R_A}{R_{MIF}}\right) - 1\right] \quad (6)$$

As mentioned in the introduction, the sum of *Eqs. 5* and *6* provides an estimation of the neuronal release rate (Rr)

$$Rr = SO + Ur \quad (7)$$

Subsequently, the ratio of uptake of released NE and neuronal release can be used as to measure the efficiency of total uptake (Eff_U)

$$Eff_U\ (\%) = \frac{Ur}{Rr} \cdot 100 \quad (8)$$

For statistical analysis, two-way ANOVA, one-way ANOVA for repeated measures with Dunnett's multiple-comparison test as a post hoc test, Student's *t*-test, and linear regression analysis were used as appropriate.

RESULTS

NE infusions. Steady-state concentrations of MIF NE were observed 120–150 min after the probes were placed and microdialysis was started. The baseline arterial plasma NE concentration was about three times lower than the MIF NE concentration ($P < 0.001$, Fig. 3). MIF NE concentrations in the LAD and LCX regions were similar, and values did not differ from those in the coronary vein. During U1 blockade, MIF NE concentration increased more than sixfold ($P < 0.01$, Fig. 3).

Circulatory and MIF NE concentrations at 20 and 30 min after the start of both systemic NE infusions did not differ, suggesting that a steady state was reached within 20 min (Fig. 3). The extraction of arterially delivered NE was 79 ± 4% and 69 ± 3% for the low and high infusion rates, respectively. Without U1 blockade, the MIF NE concentration (LAD and LCX regions) remained considerably lower than the arterial and coronary venous NE concentrations. During U1 blockade, however, the MIF NE concentration value was between the arterial plasma and coronary venous concentration values (Table 1 and Fig. 3). Without U1 blockade, the ΔMIF/ΔA ratio (*Eq. 2*) was 0.10 ± 0.01 for the lower infusion rate and 0.11 ± 0.01 for the higher NE infusion rate, whereas DMI caused the ΔMIF/ΔA ratio to rise to 0.21 ± 0.02 and 0.36 ± 0.05, respectively ($P < 0.05$).

Spillover, rate of uptake, rate of neuronal release, and the efficiency of uptake at baseline were calculated from the data of both NE infusions using *Eqs. 5–8*. Despite the large increment in circulatory NE and MIF NE concentrations, represented by the ratios of ΔNE values to baseline between the lower and higher NE doses, the kinetic parameters for NE remained unchanged (Table 2).

Systemic infusions of NE caused a marked dose-dependent increase in heart rate, blood pressure, coronary blood flow, and LV dP/dt_{max} (Table 3). The relationship between changes in LV dP/dt_{max} and MIF NE concentration was much steeper than the relationship between changes in LV dP/dt_{max} and arterial NE concentration ($P < 0.001$, Fig. 4).

Isoproterenol infusions. Systemic Iso infusions caused dose-dependent increments in arterial and venous Iso concentrations (Fig. 3 and Table 1). Circulatory and MIF Iso concentrations at 20 and 30 min after the start of both systemic Iso infusions did not differ significantly, suggesting that a steady state was reached within 20 min (Fig. 3). The extraction of arterially delivered Iso in the coronary circulation was 24 ± 5% with both the low and the high infusion rates. MIF Iso concentrations were lower than arterial and coronary venous concentrations, and values did not alter in the presence of U1 blockade. The ΔMIF/ΔA ratio for Iso was 0.37 ± 0.02 with the low infusion rate and 0.34 ± 0.02 with the high infusion rate.

Isoproterenol infusions caused dose-dependent increments in heart rate, systolic blood pressure, coronary

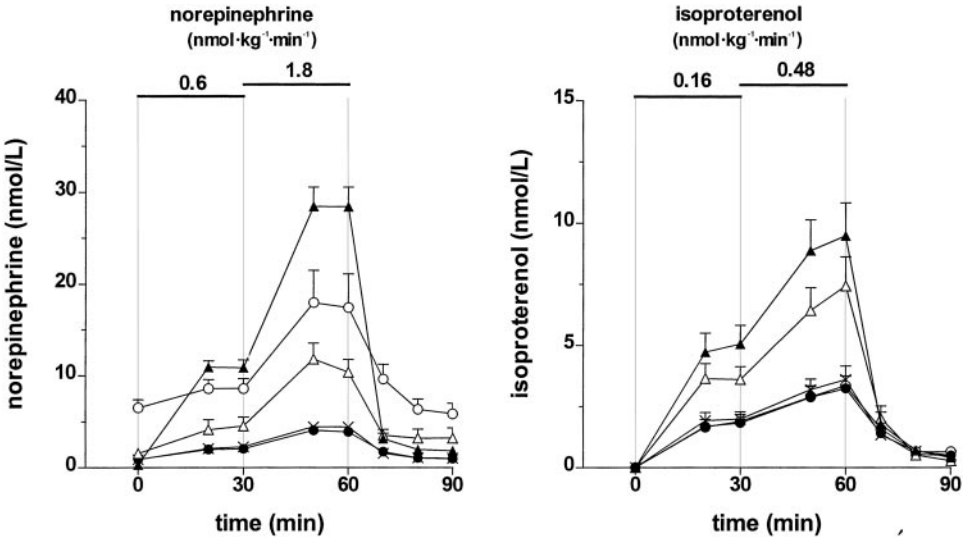

Fig. 3. NE (*left*) and Iso concentrations (*right*) during successive intravenous infusions of NE and Iso. ▲, Carotid artery; △, coronary vein; ●, myocardial interstitial fluid (MIF) left anterior descending coronary artery (LAD) region; ○, MIF LAD region under neuronal uptake (U1) blockade; ×, MIF left circumflex coronary artery (LCX) region. Data are presented as means ± SE for 8 animals.

Table 1. *Comparison of increments of circulatory and interstitial concentrations of norepinephrine and isoproterenol during intravenous infusions of norepinephrine or isoproterenol*

Location of Concentration	Norepinephrine Infusion, nmol·kg^{-1}·min^{-1}		Isoproterenol Infusion, nmol·kg^{-1}·min^{-1}	
	0.6	1.8	0.16	0.48
Carotid artery, nmol/l	10.6 ± 0.8	28.2 ± 2.1	4.9 ± 0.8	9.2 ± 2.1
Coronary vein, nmol/l	2.7 ± 0.8	9.6 ± 1.6	3.6 ± 0.6	6.9 ± 1.1
E, %	79 ± 4	69 ± 3	24 ± 5	23 ± 4
MIF LAD, nmol/l	1.1 ± 0.2	3.1 ± 0.3	1.8 ± 0.3	3.1 ± 0.5
MIF LAD + DMI, nmol/l	2.1 ± 0.5	11.2 ± 2.9†	1.8 ± 0.2	3.1 ± 0.4
MIF LCX, nmol/l	1.3 ± 0.2	3.6 ± 0.3	1.9 ± 0.3	3.4 ± 0.5
ΔMIF/ΔA	0.10 ± 0.01	0.11 ± 0.01	0.37 ± 0.02	0.34 ± 0.02
ΔMIF/ΔA + DMI	0.21 ± 0.03*	0.36 ± 0.05†	0.37 ± 0.03	0.35 ± 0.02

Values are means ± SE of norepinephrine or isoproterenol concentrations. E, cardiac arteriovenous extraction of norepinephrine; MIF, myocardial interstitial fluid; LAD, left anterior descending coronary artery; DMI, desipramine; LCX, left circumferential coronary artery; ΔMIF/ΔA, quotient of change in interstitial and arterial concentrations. *$P < 0.05$, †$P < 0.01$ compared with value in same location in absence of DMI.

blood flow, and LV dP/dt_{max} and a dose-dependent decrease in diastolic blood pressure (Table 2). The slope of the regression line of the relationship between LV dP/dt_{max} (%change) and Iso concentration was 46 ± 21 for MIF Iso and 13 ± 8 for arterial Iso concentrations.

Tyramine infusion. Tyramine, like NE, is taken up by neurons through U1, and it subsequently displaces NE from the nerve terminals because of its higher affinity for the neuronal storage proteins (37). Consequently, the degree of attenuation of tyramine-induced NE release is a measure of the degree of U1 blockade. Without U1 blockade, infusion of tyramine in the LAD caused a 15-fold rise in the MIF NE concentration in the LAD region, accompanied by a similar increase of the NE concentration in the coronary vein (Fig. 5). Under U1 blockade, this response was almost completely abolished, indicating that the blockade of U1 was virtually complete with the dose of DMI used. Tyramine infusion in the LAD was also associated with a fivefold increase in the MIF NE concentration in the LCX region. Because the systemic arterial NE concentration also slightly increased during intracoronary tyramine infusion, this increase was most likely caused by overflow of tyramine from the coronary into the systemic circulation. The hemodynamic response to tyramine was mainly confined to the heart; LV dP/dt_{max} increased almost threefold to 3,933 ± 465 mmHg/s, comparable to the increase seen during the infusions of NE and Iso.

Table 2. *Spillover, uptake, and release of norepinephrine at baseline derived from data compiled during systemic intravenous infusions of norepinephrine*

	Norepinephrine Infusion, nmol·kg^{-1}·min^{-1}	
	0.6	1.8
R_A	59.1 ± 8.2	155.3 ± 22
R_V	1.8 ± 0.3	6.9 ± 0.6
R_{MIF}	1.3 ± 0.2	3.8 ± 0.5
SO, pmol/min	35.1 ± 5.6	38.8 ± 5.4
Ur, pmol/min	194.1 ± 33.4	203.7 ± 50.8
Rr, pmol/min	229.3 ± 36.8	242.5 ± 53.4
Eff_U, %	84 ± 2	79.0 ± 2

Values are means ± SE. R_A, ratio of change in norepinephrine (ΔNE) values to baseline for arterial plasma; R_V, ratio of ΔNE values to baseline for coronary effluent; R_{MIF}, ratio of ΔNE values to baseline for MIF; SO, spillover; Ur, NE uptake from interstitium; Rr, rate of neuronal release; Eff_U, efficiency of uptake of locally released NE.

DISCUSSION

We investigated to what extent the concentration of NE in the myocardial intercellular space was related to its concentration in the arterial and coronary venous circulation at baseline and after increments in plasma or interstitial NE concentration induced by either systemic infusions of NE or an intracoronary infusion of tyramine. In addition, the importance of the U1 mechanism for the MIF NE concentration was assessed by perfusing one of the probes with the U1 inhibitor DMI and performing studies with Iso, a catecholamine that is not handled by U1. Finally, adaptation of the method introduced by Kopin et al. (22) provided an estimate of spillover, uptake, and, consequently, release of NE.

In agreement with the results of other studies using the microdialysis technique, steady-state MIF NE concentrations were observed 120–150 min after the probes were inserted and microdialysis was started. Basal MIF NE concentrations measured in this study were similar to those reported by Akiyama et al. (1, 2), who performed microdialysis in feline hearts. At baseline, MIF NE concentrations in the LCX and LAD regions were similar, suggesting no important regional differences in myocardial sympathetic activity. In contrast to various other microdialysis studies that reported arterial plasma levels at baseline to be similar to or even higher than interstitial NE levels (1, 27, 28), MIF NE concentrations in the present study were three times higher than arterial plasma concentrations. These results are in keeping with estimates made in other studies reporting that the concentration of NE at sites of release is about three- to fivefold higher than in plasma (10, 23, 26). This concentration gradient is the driving force behind the exchange of NE from the interstitial compartment to the circulation. Accordingly, one would expect this gradient to be reflected in somewhat higher NE concentrations in MIF than in the coronary vein. In contrast, NE concentrations in the coronary vein were similar to those in MIF, both under baseline conditions and during intracoronary infusion of tyramine, which induced a 15-fold increase in the MIF NE concentration. A possible explanation for this unexpected finding is that the NE concentration mea-

Table 3. *Comparison of cardiac and systemic hemodynamics during systemic intravenous infusions of norepinephrine and isoproterenol*

	Norepinephrine Infusion, nmol·kg^{-1}·min^{-1}			Isoproterenol Infusion, nmol·kg^{-1}·min^{-1}		
	Baseline	0.6	1.8	Baseline	0.16	0.48
HR, beats/min	120 ± 8	127 ± 7	141 ± 5†	125 ± 6	182 ± 4†	197 ± 7†
SAP, mmHg	103 ± 4	121 ± 4†	137 ± 5†	97 ± 5	106 ± 5*	112 ± 5†
DAP, mmHg	72 ± 5	85 ± 4†	93 ± 5†	66 ± 5	58 ± 3†	54 ± 4†
LV dP/dt_{max}, mmHg/s	1,734 ± 175	2,991 ± 202†	4,832 ± 377†	1,621 ± 325	3,810 ± 355†	5,327 ± 550†
CPF, ml/min	21 ± 3	25 ± 3†	28 ± 3†	20 ± 3	29 ± 3†	29 ± 4†
CO, l/min	2.3 ± 0.1	2.6 ± 0.2	2.9 ± 0.3†	2.2 ± 0.2	2.8 ± 0.2	3.3 ± 0.3†

Values are means ± SE. HR, heart rate; SAP, systolic aortic pressure; DAP, diastolic aortic pressure; LV dP/dt_{max}, left ventricular contractility; CPF, coronary plasma flow; CO, cardiac output. *$P < 0.05$, †$P < 0.01$, compared with baseline.

sured around the membrane of the microdialysis probe underestimated to some extent the concentration of NE at sites of release.

The observed absence of an NE gradient between the MIF and coronary vein does not support previous suggestions of the existence of an endothelial barrier for the diffusion of NE from the interstitial to the intravascular compartment (7, 25, 29, 30, 33). The presence of such an endothelial barrier could provide an explanation for the well-known difference in the relation of blood pressure response and plasma NE concentration observed for exogenously administered NE or for tyramine-induced endogenously released NE (4, 6). If an endothelial barrier for the diffusion of NE is present, MIF NE concentration and the NE concentration in the coronary vein, as a reflection of the NE concentration in the myocardial capillaries, should be different. However, both at baseline and during infusion of tyramine through the LAD, MIF NE concentrations in the LAD region and coronary vein were similar, indicating an unhindered exchange of endogenous NE from the interstitial to the vascular compartments. The considerable gradient between NE concentrations in the coronary vein and MIF that was seen during systemic infusion of NE was absent under U1 blockade and therefore is attributable to U1. Thus no endothelial barrier to the diffusion of NE appears to be present in the porcine heart.

Experimental studies and studies in humans with the use of labeled infusions of NE have shown that 60–80% of arterially delivered NE is removed by the myocardium (11, 13, 19). With the use of unlabeled NE in the present study, the extraction of NE ranged from 79 to 69%, depending on the infused dose (Table 1). Notwithstanding this high fractional removal, the MIF NE concentration remained extremely low as reflected by a ΔMIF/ΔA ratio of ~0.10. This value is close to the MIF/A ratio of 0.15 reported for the canine myocardium by Cousineau et al. (8) using the capillary-interstitium concentration model developed by Ziegler and Goresky (38). The important role of U1 in the removal of NE from the MIF was confirmed by comparing the MIF NE concentrations in the microdialysis probes in the LAD region with and without the U1 inhibitor DMI. Al-

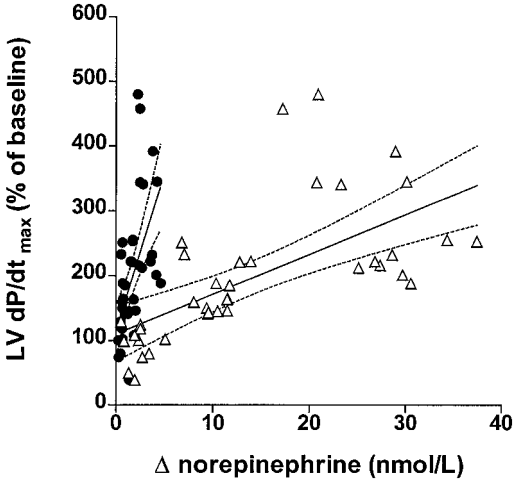

Fig. 4. Regression analysis of relationship between responses of maximum 1st derivative of left ventricular pressure (LV dP/dt_{max}) and NE concentrations in arterial blood and MIF to infusion of NE. LV dP/dt_{max} data are presented as percentages of baseline value; NE concentrations are presented as changes from baseline (ΔNE) in nmol/l. △, Carotid artery (slope = 6.1 ± 1.2, r^2 = 0.42); ●, MIF LAD region (slope = 48.5 ± 10.0, r^2 = 0.38). Dotted lines indicate 95% confidence limits. Data are presented for 8 animals.

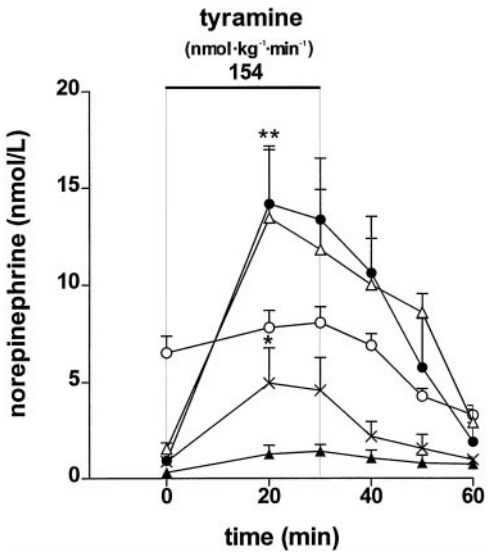

Fig. 5. NE response to an intracoronary infusion of tyramine. ▲, Carotid artery; △, coronary vein; ●, MIF LAD region; ○, MIF LAD region under U1 blockade; ×, MIF LCX region. *$P < 0.05$; **$P < 0.01$ compared with baseline. Data are presented as means ± SE for 4 animals.

though the two probes were placed no more than 1 cm apart, no interprobe interference was observed. Basal MIF NE concentrations increased more than sixfold during U1 inhibition, whereas the increases in MIF NE concentration due to infusion of NE were also markedly augmented. Local U1 inhibition in the LAD probe with DMI increased the ΔMIF/ΔA ratio to 0.21–0.36. Especially under U1 blockade, the ΔMIF/ΔA ratio for unlabeled NE is likely to be affected by NE that is released by or has leaked from the neurons. Considering this artifact, our results with DMI are quite comparable to the MIF/A ratio under U1 blockade as estimated by Cousineau et al. (8). From the differences in the ΔMIF/ΔA ratios measured in the probes with and without U1 inhibition, we calculated that 67 ± 5% of MIF NE is removed by U1 (*Eq. 3*). This value is in close agreement with values reported for the rabbit (10) but is lower than those observed in the human myocardium (12, 19).

As expected, because Iso is not taken up by U1, similar MIF Iso concentrations were measured in the probes with and without the U1 inhibitor DMI. The ΔMIF/ΔA ratios for Iso were very similar to the ΔMIF/ΔA ratios for NE during the high infusion rate of NE and local inhibition of U1 by DMI.

Because Iso is not taken up by U1, the difference in removal of NE and Iso over a certain vascular bed has been proposed to be a useful measurement of U1 activity (19, 20). Although there is some debate about the validity of the comparison of the pharmacokinetics of Iso and NE during U1 blockade (8, 9), the present findings suggest that such an approach will indeed provide a reliable estimation of U1 activity. Despite similar extractions of NE, the extraction of Iso in the porcine heart (24%) was considerably higher than that reported for the human heart (14%). This difference in extraction suggests that the cardiac extraneuronal uptake of NE is more important in the porcine than in the human heart. As proposed by Goldstein et al. (19), the proportionate fractional tissue removal of NE by U1 can be calculated by subtracting the percent removal of Iso from the percent removal of NE and dividing this difference by the percent removal of NE. With the application of this equation in the present study, it appears that ~66% of NE in the porcine myocardium is removed by U1. Although considerably lower than the value reported for the human heart (82%), this value agrees well with the proportionate fractional tissue removal of NE by U1 derived from the differences in the ΔMIF/ΔA ratios with and without local U1 inhibition.

Because of the active U1 of NE in the myocardium, the MIF concentration of NE during systemic NE infusions remained relatively low compared with the arterial NE concentration. This explains why the relationship between LV dP/dt_{max} and changes in MIF NE concentration was much steeper than the relationship between LV dP/dt_{max} and changes in arterial NE concentration. Accordingly, because Iso is not taken up by U1, the difference in relationships between LV dP/dt_{max} and interstitial or arterial Iso concentrations during Iso infusions was less distinct.

Studies performed in humans and dogs have shown that <5% of the NE that is released into the myocardial interstitium spills over into the circulation (22). In the present study, the calculated proportional spillover (~15%) was considerably higher. Because cardiac spillover in our experiments is similar to the value measured in dogs (14), it seems likely that proportional spillover was relatively high because the calculated uptake of NE was relatively low. As shown in *Eq. 6*, the calculated uptake of NE strongly depends on the ratio of R_A to R_{MIF}. Although microdialysis is a more direct technique for measuring interstitial NE concentrations than the estimates based on the isotope dilution technique, it will only provide information about the mean interstitial NE concentration and not about the concentration at sites of release. Because of the downward concentration gradient of NE from the site of entry in the interstitial fluid to the sites of uptake, R_{MIF} based on the mean interstitial NE concentration during systemic infusion of NE will be higher than R_{MIF} at the sites where uptake of NE takes place. On the basis of *Eq. 6*, overestimation of R_{MIF} will lead to the underestimation of the calculated uptake of NE.

In conclusion, microdialysis is a valuable tool for measuring as well as modifying local sympathetic activity. The present in vivo experiments largely confirm the information about NE kinetics obtained by more indirect methods. We conclude that U1 as well as extraneuronal uptake, and not an endothelial barrier, are the principal mechanisms underlying the concentration gradient of NE between the interstitial and intravascular compartments in the porcine heart.

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