Cardiac Sympathetic Nerve Stimulation Triggers Coronary t-PA Release

Jan-Arne Björkman, Sverker Jern, Christina Jern

Objective—This study was undertaken to determine whether stimulation of sympathetic cardiac nerves induces release of the thrombolytic enzyme tissue-type plasminogen activator (t-PA) in the coronary vascular bed.

Methods and Results—Anesthetized pigs were studied in an open chest model. Bilateral vagotomy was performed, and sympathetic cardiac nerves were activated by electrical stimulation (1 and 8 Hz). To evaluate possible mediating effects of increased heart rate and enhanced local blood flow, tachycardia was induced by pacing and hyperaemia by local infusion of sodium nitroprusside and clevedipine. Furthermore, to study the effects of \(\alpha\) - and \(\beta\)-adrenergic receptor stimulation, phenylephrine and isoprenaline were infused locally. In response to low- and high-frequency sympathetic stimulation, mean coronary net release of total t-PA increased approximately 6- and 25-fold, respectively. Active t-PA showed a similar response pattern. Neither tachycardia nor coronary hyperemia stimulated t-PA release. In contrast, \(\beta\)-adrenergic stimulation by isoprenaline induced an approximately 6-fold increase in coronary t-PA release, whereas no significant change in release rates occurred in response to \(\alpha\)-adrenergic stimulation by phenylephrine.

Conclusions—Stimulation of cardiac sympathetic nerves induces a marked coronary release of t-PA, and part of this response may be mediated through stimulation of \(\beta\)-adrenergic receptors. (Arterioscler Thromb Vasc Biol. 2003;23:lll-llll.)

Key Words: tissue-type plasminogen activator \(\square\) sympathetic nerves \(\square\) adrenergic \(\square\) coronary \(\square\) pigs

Tissue-type plasminogen activator (t-PA) is the main fibrinolytic activator in the vascular compartment.\(^1,2\) The endothelium continuously secretes t-PA by a constitutive pathway. In addition, t-PA can be rapidly released from endothelial stores in response to various stimuli by a regulated pathway. Such an acute release of t-PA is a key event in initiating an endogenous fibrinolytic response,\(^2\) which in turn may result in endogenous removal of thrombi. Unfortunately, however, regulated release of t-PA in vivo cannot be determined by measurements of systemic plasma levels of t-PA, because plasma t-PA is sensitive to alterations in hepatic blood flow.\(^3,5\) To overcome this problem, we adapted the human perfused-forearm model to study local t-PA release\(^6,7\) and demonstrated that both mental stress and local infusion of norepinephrine induce an acute release of t-PA in the forearm vascular bed.\(^6,8\)

It is well-known that sympathetic stimulation induces activation of procoagulant mechanisms on the systemic level.\(^9,10\) It is therefore of interest to investigate if an acute t-PA release can be induced also in the coronary circulation by sympathetic activation, because such a response might oppose stress-induced procoagulant activation and thereby protect against clot formation. In the present study, we investigated the effect of sympathetic stimulation on coronary t-PA release in a pig model by determining coronary release of t-PA during electrical stimulation of cardiac sympathetic nerves. If such a stimulatory effect could be demonstrated, a second aim was to investigate if the accompanying hemodynamic alterations that occur during sympathetic stimulation (ie, tachycardia and coronary hyperaemia) as such could mediate the response. We also examined the effects of local infusion of the unselective \(\beta\)-adrenergic agonist isoprenaline and the \(\alpha\)-adrenergic agonist phenylephrine on coronary t-PA release.

Methods

Animals
The study was performed on 29 healthy, young Swedish farm pigs of either sex. Their body weight ranged from 25 to 35 kg. They were maintained on a standard diet but were fasted with free access to water 12 hours before the study. Animal care and handling conformed with the Guide for the Care and Use of Laboratory Animals, published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Committee for Ethical Review of Animal Experiments at the University of Göteborg approved the protocols.

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Preparations and Experimental Protocols

Experiment I: Stimulation of Cardiac Sympathetic Nerves

The hypothesis that activation of sympathetic nerves induces regulated release of t-PA was tested in 7 pigs. Anesthesia was induced by intramuscular injection of 20 mL Saffan (alphaxalone 9% wt/vol and alphadoline acetate 0.3% wt/vol) and maintained by a continuous intravenous infusion of Saffan (3 mL/h) and α-chloralose (90%, Aldrich-Chemie) given as a bolus of 40 mg/kg followed by 20 mg/kg per h. An orotracheal tube was introduced, and volume-controlled mechanical ventilation was instituted (Servo Ventilator 900C, Siemens Elema).

After bilateral vagotomy, the right and left stellate ganglia were carefully isolated from connective tissue. Each of the 2 ansae subclaviae were attached to a silver ring electrode, which was isolated and connected to a computerized stimulator. After median sternotomy, a shunt from a coronary vein to the right auricle of the heart was established. To that end, the coronary vein running parallel to the left anterior descending artery (LAD), draining the ventral part of the heart corresponding to the area supplied by LAD, was gently exposed. A 5- to 10-mm segment of the vein approximately 80 mm from the apex was separated carefully and cannulated retrogradely with a PP 260 polyethylene catheter (Portex). The catheter was fixed to the vein by a ligature, and the other end was inserted into the right auricle. The shunt permitted rapid blood sampling from the coronary vein and facilitated exact timing of the sampling procedure relative to the start of stimulation. Immediately after establishment of the shunt, the animals were heparinized (bolus 5000 IU followed by 50 IU/kg per h). Blood flow in the vein-auricle line was monitored with an ultrasonic probe (Transonic Ultrasonic Transit-Time). Because of technical difficulties, 1 animal only received unilateral stimulation (right side).

An equilibration period of at least 60 minutes was allowed after the surgical preparation before the actual experiment started with baseline recordings for 10 minutes. Stimulation of sympathetic nerves was induced by supramaximal current, ie, 5 to 15 mA with a pulse duration of 2 ms (Simultis Isolator A 385, World Precision Instruments, driven by a custom-designed computer system, AstraZeneca) to recruit all nerve fibers. Continuous stimulation was performed during 3 minutes with single impulses at 1 and 8 Hz to study the effect of mild and submaximal sympathetic stimulation, respectively. Each animal was left to recover for at least 30 minutes between stimulations.

Experiments II and III: Effects of Tachycardia and Hyperemia

The possibility that increased heart rate or enhanced coronary blood flow contributed to the observed regulated release of t-PA was explored by stimulation in an additional series of experiments. Seven pigs were exposed to right atrial pacing and local coronary infusion of the NO-donor nitroprusside (sodium nitroprusside, Merck). Animals were premedicated by an intramuscular injection of 2 mg/kg of midazolam (Dormicium 5 mg/mL, Roche) and 10 mg/kg of ketamine hydrochloride (Ketaminol 100 mg/mL, Vetpharma AB). Twenty minutes later, an intravenous infusion of 2 to 5 mg/kg of propofol (Diprivan 10 mg/mL, ZENEGA Limited) was started. This was followed by 100 mg/kg of α-chloralose as an intravenous bolus injection and then 25 to 50 mg/kg per hour as a continuous infusion. The reason that a different anesthesia was used compared with the first series was that Saffan was no longer available on the market at this time.

After sternotomy, a 5- to 8-mm segment of the LAD was carefully isolated 50 to 80 mm from apex. A Transonic flow probe (1.5 mm, Transonic System Inc) was placed around the LAD to measure coronary blood flow. By the time of this second experimental series, we had developed an alternative simplified procedure to obtain samples from the coronary vein at precisely defined time intervals. To this end, a custom-designed needle (20-gauge) attached to a thin polyethylene catheter (PE 90 Intramedic, Clay Adams) was used. This needle was placed in the local coronary vein accompanying the LAD for intraand coronary infusions, a 16-gauge needle with side holes near the tip was connected to a polyethylene catheter (PE 50 Intramedic, Clay Adams) and inserted into the LAD distal to the flow probe. For pacing of the heart, a bipolar clip electrode was attached to the right atrial appendage.

After surgical preparation, an equilibration period of at least 30 minutes was allowed before the experiment started with baseline recordings for 10 minutes. The heart was then paced during 3 minutes at 150 and 200 bpm, respectively. After an additional 30-minute recording period and a 10-minute baseline recording period, sodium nitroprusside (SNP) was infused into the coronary artery through 2 dose steps, 0.1 and 0.5 mg/min during 3 minutes each.

Because we could not achieve an enhanced coronary blood flow by local administration of SNP without inducing systemic effects, an additional series of experiments was performed with the ultra-short-acting, vascular selective dihydropyridine calcium antagonist clevedipine (AstraZeneca). Because the half-life of clevedipine is only approximately 12 seconds, we have earlier been able to enhance coronary blood flow with this substance without inducing systemic effects. Seven animals were included, and surgical preparation, equilibration period, and baseline recordings were as in experiment II. Clevedipine was infused into the coronary artery at a constant rate over 3 minutes. Blood samples were collected in syringes containing 1/10 0.45 mol/L sodium citrate buffer, pH 4.3. Plasma was isolated within 15 minutes.
by centrifugation at 5000g for 15 minutes at 4°C. Plasma samples were immediately frozen and stored at −70°C.

**Measurements of Plasma t-PA**

Plasma levels of total t-PA antigen were determined by an enzyme-linked immunoassay (TimElize t-PA, catalogue No. 1105, Biopool AB) that detects free and complexed t-PA with equal efficiency. Calibration was performed with porcine t-PA diluted in t-PA-depleted porcine plasma as earlier described. Active t-PA was determined with a spectrophotometric parabolic rate assay (Spec-trolyseTM/fibrin t-PA, catalogue No. 101101, Biopool AB). By quenching with polyclonal goat anti-porcine t-PA IgG (catalogue No. 105301, Biopool AB), we have earlier shown that this assay is specific for t-PA. Human single-chain t-PA calibrated against the International Standard for t-PA (World Health Organization’s First International Standard for t-PA coded 88/670) from the National Institute for Biological Standards and Control, Hertfordshire, England) was used as standard in this assay. Thus, in the following, t-PA activity is expressed in units, with 1 unit of porcine t-PA being equivalent in the employed assay to 1 international unit of human t-PA. Samples from each experimental animal were analyzed on 1 single microtiter plate. All samples were analyzed in duplicate, and mean intra-assay coefficients of variation were 2.5% and 3.5% for total and active t-PA, respectively.

**Calculation of Coronary Net Release of t-PA**

Individual venoarterial concentration gradients were obtained by subtraction of the values measured in simultaneously collected venous and arterial plasma. Blood flow was interconverted to plasma flow using individual arterial hematocrits, and net release rate was defined as the venoarterial concentration gradient times plasma flow per unit of time across the coronary vasculature, as described.

**Statistical Analysis**

Standard statistical methods were used. All results are presented as mean and SEM. Wilcoxon’s signed rank sum test was used to evaluate responses of t-PA variables to sympathetic stimulation. Responses to cardiac pacing and coronary infusions were evaluated by two-way ANOVA and nonparametric Friedman’s test hemodynamic and t-PA variables, respectively. Post hoc analysis was performed by paired Student’s t test and Wilcoxon’s signed rank sum test. Statistical tests were considered significant at \( P < 0.05 \).

**Results**

**Stimulation of Cardiac Sympathetic Nerves**

(Experiment I)

Cardiac sympathetic nerve stimulation caused an instant hemodynamic response that remained constant throughout the stimulation period (Figure 1). HR increased by approximately 40% and 70% and MAP by 15% and 30% in response to low- and high-frequency sympathetic stimulation, respectively. Coronary blood flow increased approximately 2- and 2.5-fold.

At prestimulation baseline, there was a significant step up from the arterial to venous side across the coronary vascular bed for both total and active t-PA (Table 1). The average prestimulation coronary net release rate of total and active t-PA was 7 ng/min (range, 1 to 30 ng/min) and 13 U/min (range, 4 to 22 U/min), respectively (Figure 2). A marked increase in net release rate of both total and active t-PA was observed in response to both stimulation frequencies, but this increase was only statistically significant in response to high-frequency stimulation (Figure 2). Average total t-PA release rates amounted to 40 ng/min (range, 14 to 86 ng/min) and 179 ng/min (range, 11 to 351 ng/min) during low- and high-frequency stimulation, respectively. The corresponding figures for active t-PA were 53 U/min (range, 6 to 111 U/min) and 132 U/min (range, 11 to 303 U/min). No t-PA response was observed in the animal that received unilateral stimulation, and if this animal was excluded, total mean t-PA release rates amounted to 45 and 207 ng/min (range, 11 to 351 ng/min) during low- and high-frequency stimulation, respectively. After cessation of stimulation, net release rates of t-PA rapidly returned to prestimulation levels (Figure 2). Regarding systemic plasma levels, a significant increase in the arterial plasma level of total t-PA was observed during high-frequency stimulation (Table 1).

**Effects of Tachycardia and Hyperemia**

(Experiments II and III)

To evaluate whether increased HR could contribute to the observed t-PA response, the heart was paced to similar rates as obtained during low- and high-frequency stimulation, ie, approximately 150 and 200 bpm, respectively. There was a slight reduction in the venous plasma level of t-PA, but no significant alterations in coronary blood flow, arterial plasma levels, or net release of total t-PA were observed in response to either stimulation frequency (Table 2).
To examine if t-PA release was flow dependent, we first infused SNP, and no significant effect on net release of total t-PA was observed (Table 2). Both doses of SNP induced a 1.5-fold increase in coronary blood flow compared with the 2-fold increase observed in response to high-frequency sympathetic stimulation. However, a significant drop in MAP occurred already at the low dose, and MAP was reduced even further in response to the high dose (Table 2). Thus, it was not possible to enhance local coronary blood flow further by local infusion of SNP without inducing substantial systemic effects. We therefore performed an additional series of experiments in which coronary blood flow was enhanced by local infusion of the ultra–short-acting calcium-antagonist clevedipine. In contrast to SNP, clevedipine induced a dose–dependent increase in coronary blood flow without any significant systemic effects (Table 3). The mean increase in

### Table 1. Effects of Stimulation of Cardiac Sympathetic Nerves (1 and 8 Hz) on Plasma Concentrations of t-PA

<table>
<thead>
<tr>
<th></th>
<th>Before Stimulation</th>
<th>During Stimulation</th>
<th>After Stimulation, 1 min</th>
<th>After Stimulation, 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial total t-PA, ng/mL</td>
<td>6.60 (1.22)</td>
<td>7.70 (2.00)</td>
<td>8.26 (2.08)</td>
<td>8.15 (2.54)</td>
</tr>
<tr>
<td>Venous total t-PA, ng/mL</td>
<td>7.57 (0.72)</td>
<td>10.3 (2.08)</td>
<td>8.95 (2.41)</td>
<td>9.13 (2.99)</td>
</tr>
<tr>
<td>V-A gradient total t-PA, ng/mL</td>
<td>0.97 (0.60)</td>
<td>2.58 (0.65)</td>
<td>0.70 (0.40)</td>
<td>0.83 (0.40)</td>
</tr>
<tr>
<td>Arterial active t-PA, U/mL</td>
<td>2.29 (0.62)</td>
<td>3.17 (1.31)</td>
<td>4.23 (1.74)</td>
<td>3.51 (1.43)</td>
</tr>
<tr>
<td>Venous active t-PA, U/mL</td>
<td>3.88 (0.82)</td>
<td>6.45 (1.68)</td>
<td>5.02 (1.73)</td>
<td>4.44 (1.80)</td>
</tr>
<tr>
<td>AV-gradient active t-PA, U/mL</td>
<td>1.20 (0.24)</td>
<td>3.16 (0.97)</td>
<td>0.78 (0.29)</td>
<td>0.93 (0.53)</td>
</tr>
</tbody>
</table>

Values shown are mean (SEM).

### Table 2. Effects of Stimulation of Cardiac Sympathetic Nerves (1 and 8 Hz) on Plasma Concentrations of t-PA

<table>
<thead>
<tr>
<th></th>
<th>Before Stimulation</th>
<th>During Stimulation, 1 Hz</th>
<th>After Stimulation, 1 min</th>
<th>After Stimulation, 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial total t-PA, ng/mL</td>
<td>8.37 (1.98)</td>
<td>9.19 (2.02)*</td>
<td>9.39 (2.10)</td>
<td>10.0 (2.48)</td>
</tr>
<tr>
<td>Venous total t-PA, ng/mL</td>
<td>9.48 (2.33)</td>
<td>16.0 (3.06)†</td>
<td>10.7 (2.66)</td>
<td>12.4 (3.81)</td>
</tr>
<tr>
<td>V-A gradient total t-PA, ng/mL</td>
<td>1.11 (0.43)</td>
<td>6.78 (1.39)*</td>
<td>1.34 (0.59)</td>
<td>2.38 (1.43)</td>
</tr>
<tr>
<td>Arterial active t-PA, U/mL</td>
<td>3.47 (1.31)</td>
<td>3.77 (1.46)†</td>
<td>4.32 (1.68)</td>
<td>3.14 (1.57)</td>
</tr>
<tr>
<td>Venous active t-PA, U/mL</td>
<td>4.68 (1.77)</td>
<td>8.93 (2.21)†</td>
<td>5.17 (1.86)</td>
<td>4.18 (2.10)</td>
</tr>
<tr>
<td>AV-gradient active t-PA, U/mL</td>
<td>0.58 (0.43)</td>
<td>4.26 (1.41)*</td>
<td>0.65 (0.37)</td>
<td>1.70 (0.85)</td>
</tr>
</tbody>
</table>

Values shown are mean (SEM).

To examine if t-PA release was flow dependent, we first infused SNP, and no significant effect on net release of total t-PA was observed (Table 2). Both doses of SNP induced a 1.5-fold increase in coronary blood flow compared with the 2-fold increase observed in response to high-frequency sympathetic stimulation. However, a significant drop in MAP occurred already at the low dose, and MAP was reduced even further in response to the high dose (Table 2). Thus, it was not possible to enhance local coronary blood flow further by local infusion of SNP without inducing substantial systemic effects. We therefore performed an additional series of experiments in which coronary blood flow was enhanced by local infusion of the ultra–short-acting calcium-antagonist clevedipine. In contrast to SNP, clevedipine induced a dose–dependent increase in coronary blood flow without any significant systemic effects (Table 3). The mean increase in
coronary blood flow was 2.5-fold at the highest dose, ie, a similar relative change as observed in response to high-frequency stimulation. However, there was no significant alteration in coronary t-PA release (Table 3).

Effects of Local Myocardial \(\alpha\)- and \(\beta\)-Adrenergic Stimulation (Experiment IV)

IPR induced a dose-dependent increase in both HR and coronary blood flow (Table 4). IPR also induced a significant increase in the venous plasma concentrations of t-PA as well as an increase in coronary net release of t-PA that approached statistical significance (\(P=0.06;\) Table 4). In addition, the arterial plasma concentration of t-PA increased, which indicates that IPR also had a systemic effect. Mean maximal increase in coronary net t-PA release in response to IPR was 6-fold, compared with 6- and 25-fold in response to low- and high-frequency sympathetic stimulation, respectively. In contrast to IPR, PE had no significant effects on either hemodynamic variables or coronary t-PA release (Table 4).

Discussion

The present study demonstrates for the first time that stimulation of sympathetic cardiac nerves induces an acute release of t-PA. Most of the net release of t-PA is in its free, active form, indicating that local sympathetic stimulation has no major effect on the inhibitors of t-PA. We also demonstrate that the t-PA response is independent of changes in heart rate and in local blood flow per se, because neither pacing nor local infusions of vasodilatory agents induced any acute release of t-PA.

The advantage with the model we describe here is that it permits determination of instantaneous local release of t-PA from the coronary vascular bed without any confounding effect of changes in hepatic clearance. The present results corroborate with our previous findings of a release of t-PA in response to acute mental stress across the forearm vascular bed in humans.6,8 The present study also shows that stimulation of sympathetic cardiac nerves induces a substantial regulated release of t-PA in the coronary vascular bed. A 20-fold relative increase in coronary t-PA release was observed during high-frequency stimulation, which is of the same order of magnitude as the response we and others have observed during local administration of different pharmacological agonists in the human forearm.7,8,18–23 Responses of similar magnitude were also recently reported when the...
human coronary vascular bed was stimulated by infusion of substance P and bradykinin.\textsuperscript{24,25}

Several mechanisms may be involved in the observed release of t-PA in response to stimulation of sympathetic nerves. Because neither pacing nor local infusion of vasodilatory agents enhanced coronary t-PA release rates, it is unlikely that the response was attributable to mechanical factors acting on the vessel wall by changes in flow or pulsatility. However, in these mechanistic studies, it was not possible to produce a similar relative increase in flow by local infusion of SNP as that induced by high-frequency stimulation without concomitant systemic effects. We therefore also evaluated the effect of increased flow as induced by the ultra–short-acting Ca\textsuperscript{2+} antagonist clevedipine, although one limitation of this approach is that regulated release of t-PA is dependent on Ca\textsuperscript{2+} influx.\textsuperscript{26,27} However, the present data, taken together with several earlier studies showing that SNP is not a stimulus for regulated release of t-PA either in the human forearm or in the human coronary vascular bed,\textsuperscript{7,18–20,22,24} indicate that the enhanced blood flow could not explain the observed profound release of t-PA in response to sympathetic stimulation. Catecholamines are known to enhance systemic levels of P and bradykinin.\textsuperscript{24,25}

### TABLE 4. Effects of Local Infusion of Isoprenaline (IPR) and Phenylephrine (PE) on Hemodynamics and Coronary t-PA Release (n=8)

<table>
<thead>
<tr>
<th>Basal</th>
<th>IPR I</th>
<th>IPR II</th>
<th>IPR III</th>
<th>IPR IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>131 (18)</td>
<td>143 (21)</td>
<td>142 (22)</td>
<td>135 (21)</td>
</tr>
<tr>
<td>Coronary artery flow, mL/min</td>
<td>32 (5)</td>
<td>34 (6)</td>
<td>35 (6)</td>
<td>32 (5)</td>
</tr>
<tr>
<td>Arterial total t-PA, ng/mL</td>
<td>7.8 (1.5)</td>
<td>7.6 (1.4)</td>
<td>8.0 (1.7)</td>
<td>7.9 (1.3)</td>
</tr>
<tr>
<td>Venous total t-PA, ng/mL</td>
<td>7.8 (1.3)</td>
<td>7.9 (1.5)</td>
<td>8.5 (1.5)</td>
<td>8.2 (1.6)</td>
</tr>
<tr>
<td>V-A gradient t-PA, ng/mL</td>
<td>0.1 (0.3)</td>
<td>0.2 (0.3)</td>
<td>0.4 (0.4)</td>
<td>0.2 (0.3)</td>
</tr>
<tr>
<td>Coronary net release total t-PA, ng/min</td>
<td>0.5 (5.0)</td>
<td>10.6 (7.5)</td>
<td>18.7 (13.4)</td>
<td>44.8 (11.7)*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Basal</th>
<th>PE I</th>
<th>PE II</th>
<th>PE III</th>
<th>PE IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>86 (6)</td>
<td>82 (4)</td>
<td>80 (7)</td>
<td>82 (6)</td>
</tr>
<tr>
<td>Coronary artery flow, mL/min</td>
<td>25 (4)</td>
<td>36 (8)</td>
<td>40 (9)*</td>
<td>52 (11)*</td>
</tr>
<tr>
<td>Arterial total t-PA, ng/mL</td>
<td>5.8 (0.9)</td>
<td>6.3 (1.1)*</td>
<td>7.0 (1.2)*</td>
<td>7.3 (1.2)*</td>
</tr>
<tr>
<td>Venous total t-PA, ng/mL</td>
<td>6.9 (1.0)</td>
<td>7.7 (1.3)*</td>
<td>7.9 (1.3)*</td>
<td>8.4 (1.2)*</td>
</tr>
<tr>
<td>V-A gradient t-PA, ng/mL</td>
<td>1.1 (0.7)</td>
<td>1.0 (0.6)</td>
<td>0.8 (0.4)</td>
<td>1.1 (0.2)</td>
</tr>
<tr>
<td>Coronary net release total t-PA, ng/min</td>
<td>8.9 (3.4)</td>
<td>45.3 (27.8)</td>
<td>29.6 (13.0)</td>
<td>44.8 (11.7)*</td>
</tr>
</tbody>
</table>

**Values shown are mean (SEM).**

**Statistical analysis as in Table 3.** *P<0.05; †P<0.01; ‡P<0.001**

However, previous measurements of systemic plasma levels of t-PA,\textsuperscript{10} as well as data obtained by computer simulation to model t-PA secretion,\textsuperscript{3} support our conclusion that \(\beta\)-adrenergic receptors mediate release of t-PA. Results from isolated perfused vascular systems also indicate that regulated release of t-PA is induced by \(\beta\)-adrenergic receptor stimulation.\textsuperscript{29,30} and it was recently shown that isoproterenol is a stimulus for t-PA release in the human forearm.\textsuperscript{22} Hence, a plausible mechanism is that activation of cardiac sympathetic nerves increases local norepinephrine spillover, which in turn induces a regulated release of t-PA from endothelial stores. However, in the present study, it was not possible to enhance t-PA release rates by infusion of isoprenaline to similar levels as obtained during sympathetic stimulation, presumably because of lesser local concentrations of isoprenaline by the infusion than the local norepinephrine concentration obtained during stimulation sympathetic cardiac nerves.

Another possibility is that t-PA released from the sympathetic nervous system itself contributed to the observed response. O’Rourke and colleagues\textsuperscript{32} have shown that sympathetic neurons can synthesize and release t-PA. Interestingly, the same group recently reported that sympathetic stimulation...
in rats greatly reduced steady-state plasma t-PA activity in intact animals as well as t-PA release from isolated whole-vessel explants,33 which may suggest that the sympathetic nervous system contributes to plasma t-PA.

One limitation of the present study is that electrical activation of sympathetic cardiac nerves is an experimental stimulus. However, data from our laboratory show that even more pronounced sympathoadrenal stimulation, as judged by the 20-fold increase in t-PA release rates in response to high-frequency stimulation. This release could not be obtained by the concomitant hemodynamic alterations, because pacing-induced tachycardia and local hyperaemia induced by 2 different vasodilators did not cause an augmented t-PA release. However, by stimulation of the β-adrenoceptors by intracoronary isoprenaline infusion, an acute t-PA release was induced.

Acknowledgments

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