Effect of Regional Myocardial Ischemia on Sympathetic Nervous System as Assessed by Fluorine-18-Metaraminol

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With the introduction of radiolabeled catecholamine analogues, the noninvasive evaluation of the cardiac sympathetic nervous system has become possible. This study evaluated the effect of regional ischemia on myocardial retention of the new norepinephrine analogue 6-[^{18}F] fluorometaraminol (FMR) in the open chest dog model. Six dogs were injected intravenously with FMR following 30-min occlusion of the left anterior descending artery. Six sham animals served as control group. Regional myocardial blood flow as determined by microspheres decreased 87% during ischemia ($p < 0.01$), but was not significantly different from control myocardium following reperfusion. Regional myocardial ^{18}F activity as determined postmortem was significantly reduced in reperfused myocardium (−34%), which paralleled an 18% reduction of tissue norepinephrine concentration. Thus, short time periods of coronary occlusion affect neuronal function indicating the sensitivity of the sympathetic nerve terminals to ischemia. FMR provides a new tracer approach for the characterization of neuronal integrity in postischemic myocardium.

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Despite the improved therapeutic management of acute and chronic ischemic heart disease, sudden death contributes significantly to the mortality of patients with coronary artery disease (*1,2*). Impaired left ventricular function following myocardial infarction has been shown to be an important prognostic indicator for subsequent cardiac events (*3*). However, the mortality in this group of patients is less related to the consequences of heart failure than to acute dysrhythmia (*4,5*). Experimental investigations suggest a significant role of the sympathetic nervous system in the development of dysrhythmia in ischemic heart disease (*6–10*). Several studies have shown regional myocardial denervation following acute myocardial infarction (*11,12*). The area of denervation exceeded the area of tissue necrosis generating heterogeneous sympathetic innervation throughout viable myocardium. Animal studies indicate the increased sensitivity of ischemically denervated myocardium to electrophysiologic stimulation (*13*). In addition, several clinical studies have demonstrated the beneficial effect of beta receptor blockade for survival following acute myocardial infarction, indirectly suggesting the involvement of the sympathetic nervous system in the induction of lethal dysrhythmia (*14,15*).

The integrity of the sympathetic nerve terminals is necessary for the regulation of norepinephrine concentration in myocardial tissue (*16*). The reuptake of catecholamines by sympathetic nerve terminals is an important mechanism in reducing extravascular norepinephrine concentration (*17*). Rapid removal of catecholamines from the extravascular space protects the postsynaptic receptor sites from overexposure to circulating catecholamines and, hence, may play an important role in the regulation of regional sympathetic tone (*18*).

With the recent introduction of radiolabeled catecholamine analogues, uptake of catecholamines in sympathetic nerve endings can be studied by imaging techniques. Metaiodobenzylguanidine (MIBG) has been employed as a single-photon emitting imaging tracer for the evaluation of the adrenergic system in various organs including the heart (*20*). However, SPECT technology provides only qualitative information of regional tracer distribution in the heart. 6-[F-18]fluorometaraminol (FMR) is a new norepinephrine analogue developed for positron emission tomography (*21*). The retention of FMR in the regionally denervated canine heart closely parallels tissue norepinephrine content (*22*). Pharmacologic blocking studies in vivo support the neuronal localization of FMR by uptake I carrier system and vesicular storage (*23,24*).

The purpose of this study was to define the sensitivity of sympathetic nerve terminals to short time periods of

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ischemia using this new radiopharmaceutical. Regional FMR retention in the canine myocardium was assessed under control conditions and following 30 min of regional ischemia. In addition, the regional radiotracer concentration was related to myocardial blood flow and norepinephrine tissue content in the normal and post-ischemic canine myocardium.

MATERIALS AND METHODS

Fourteen mongrel dogs of both gender, weighing from 15 to 25 kg were studied. The animals were anesthetized with pentobarbital (30 mg/kg i.v.), intubated and ventilated with a mixture of room air and oxygen to maintain partial plasma oxygen pressure over 100 mmHg. A left thoracotomy was performed in the fifth intercostal space and the heart suspended in a pericardial cradle. Catheters were inserted in both femoral arteries and the left atrium for monitoring of blood pressure, withdrawal of arterial blood and for the injection of radiolabeled microspheres. The left anterior descending artery (LAD) was isolated and a snare applied for subsequent occlusion. Following surgery and instrumentation the animals were allowed to recover for 30 min.

Radiosynthesis

FMR was synthesized using the fluoro demercuracation method of Mislankar et al. (*21*). Specific activity of FMR ranged from 1 to 10 Ci/mmol.

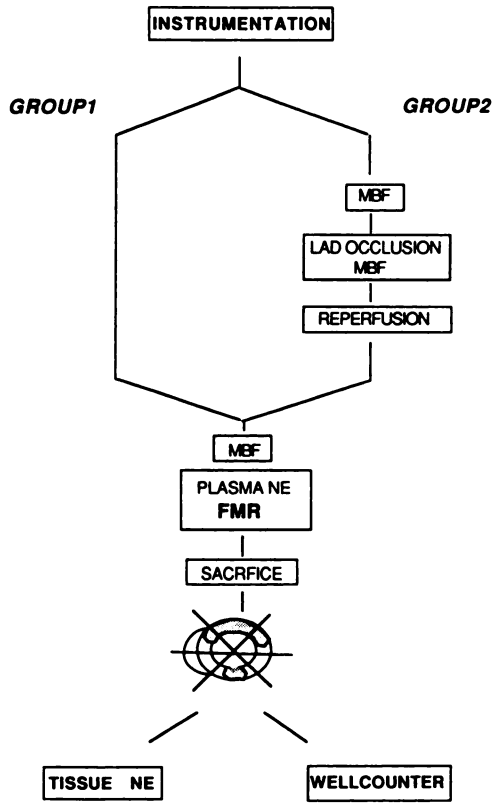

FIGURE 1
Study protocol.

Study Protocol

The study protocol consisted of two experimental groups (Fig. 1). Six Group 1 animals served as controls. No occlusion of the coronary artery was performed in this group. In eight Group 2 animals, the LAD was occluded for 30 min followed by slow reperfusion.

Regional myocardial blood flow was determined using the microsphere technique (*25*). Microspheres labeled with cerium-141, tin-113, and scandium-123 (Du Pont, N. Billerica, MA) were injected into the left atrium in random order at baseline, during occlusion, and 30 min after reperfusion. During each microspheres injection, arterial blood was withdrawn from the femoral artery at a rate of 7.12 ml/min using a Harvard pump (Gould Electronics, Inc., Valley View, OH).

FMR was injected intravenously in Group 1 animals 1 hr after instrumentation, and in Group 2 animals 30 min after reperfusion. For the definition of the arterial ^{18}F input function, arterial blood samples were obtained for 30 min from time of FMR injection with high temporal resolution.

Thirty minutes after FMR injection the animals were killed with an overdose of pentobarbital. Prior to killing, the LAD was occluded in all animals and blue dye (monastrel blue) injected into the left atrium in order to delineate the vascular territory distally to LAD occlusion. The hearts were immediately excised and cut into four slices perpendicular to the long axis of the left ventricle. Each slice was divided into sectorial sections and the stained tissue counted for ^{18}F and microsphere activity using a gamma-scintillation counter (Packard Instruments, Downers Grove, IL.). Regional tissue ^{18}F activity was divided by the integral of ^{18}F activity in blood and expressed in arbitrary units.

Norepinephrine plasma levels were determined at baseline, during occlusion and prior FMR injection. Norepinephrine tissue concentration was determined from representative samples in the center of the LAD territory as well as in the posterior myocardium. Plasma and tissue norepinephrine content were determined using the electrochemical high-performance liquid chromatography (HPLC) technique and expressed in pg/ml and ng/g of tissue, respectively (*26*).

RESULTS

Twelve out of 14 animals completed the study protocol. One dog developed ventricular fibrillation during the early reperfusion period which was promptly treated by electrical defibrillation.

Regional myocardial blood flow under control conditions was not significantly different in the anterior and posterior wall of the left ventricle (Table 1). There was a significant decrease in regional myocardial blood flow during occlusion of the LAD coronary artery in Group 2 animals. The relative blood flow reduction in the ischemic segments averaged 87% ± 11% ($p < 0.001$). Blood flow following reperfusion returned to 81% of control in Group 2 animals (NS) (Table 1).

Figure 2 shows representative examples of the two experimental groups relating regional myocardial flow to FMR retention in one midventricular cross-section. In the Group 1 animal, there was homogeneous blood flow averaging ~ 1 ml/min/g of tissue which paralleled

TABLE 1
Regional Blood Flow (ml/min/g)

	Baseline	Occlusion	Reperfusion
Group I			
Ant.	1.31 ± 0.2	—	—
Post.	1.41 ± 0.4	—	—
Group II			
Ant.	1.14 ± 0.4	0.18 ± 0.2*	1.04 ± 0.4
Post.	1.32 ± 0.5	1.41 ± 0.5	1.29 ± 0.3

*$p < 0.001$.
Post. = posterior and Ant. = anterior.

homogeneous FMR retention. The data in the Group 2 animal revealed decreased FMR retention in the reperfused segment which exceeded the regional reduction of myocardial blood flow. The measurements of FMR retention and blood flow in normal and reperfused myocardium of the two animal groups are summarized in Table 2. In the control animals, there was a small (7%) but significant reduction of FMR retention in the anterior wall (Table 3). In Group 2 animals, the FMR retention at 30 min after tracer injection was reduced by 38% ($p < 0.01$), while FMR retention in the remote myocardium was not significantly different from control animals in Group 2.

Tissue norepinephrine content was determined in four control animals and five Group 2 animals. No significant difference in the control animals was observed between anterior and posterior walls of the left ventricle (Fig. 3). In the interventional animals, regional norepinephrine content in the reperfused myocardium was reduced by 18% as compared to the posterior wall ($p < 0.05$). There was only a weak but significant correlation of the individual tissue norepinephrine content and regional FMR retention ($r = 0.33$).

Determination of plasma norepinephrine concentration in Group 1 and Group 2 animals prior to FMR injection did not demonstrate significant differences between Group 1 and Group 2 animals. Correlation of plasma norepinephrine levels and regional FMR uptake in the normal and reperfused segment did not reveal a significant relationship between both measurements.

DISCUSSION

The data of this study indicate that 30 min regional myocardial ischemia leads to sustained abnormalities of FMR retention in reperfused myocardium suggesting decreased catecholamine retention in sympathetic nerve terminals. These observations confirm previous reports about the depletion of myocardial tissue norepinephrine content in ischemically injured myocardium as assessed by in vitro techniques and indicate the potential of FMR in combination with PET to provide the noninvasive evaluation of sympathetic nervous system in ischemic heart disease.

Methodical Consideration

FMR is a newly synthesized norepinephrine analogue (*21*). Previous animal studies in the rat and dog model have shown that the myocardial retention can be blocked by neurotoxines and by pharmacologic inhibition of the U I uptake as well as the vesicular storage mechanism (*23*). The neurotoxine 6-hydroxydopamine reduced myocardial retention of FMR by 76%, while desipramine (uptake I inhibition) and reserpine (storage

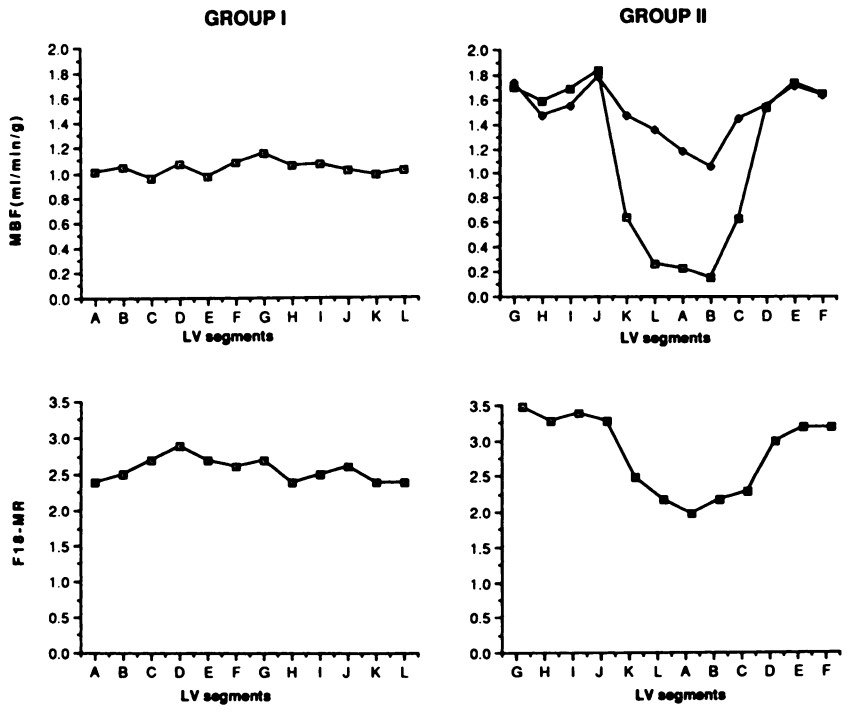

FIGURE 2
Examples of Group I and Group II regional myocardial blood flow (above) and ^{18}F activity displayed as an unrolled cross-section. Segments 1 and 12 represent posterior wall of the left ventricle while segments 4–6 are tissue sections of the anterior wall. Note the homogeneous distribution of flow and ^{18}F activity in the control animal. The Group II animal was successfully reperfused as evident by the blood flow measurements. Regional ^{18}F activity remains reduced in the reperfused segments.

TABLE 2
Regional [^{18}F]metaraminol Retention and Tissue Norepinephrine Content (ng/g)

	^{18}F	Norepinephrine
Group I		
Ant.	28.3 ± 3.8*	441 ± 144
Post.	30.5 ± 4.5	431 ± 99
Group II		
Ant.	21.1 ± 17.1†	297 ± 96*
Post.	31.9 ± 21.3	362 ± 103

* $p < 0.05$.
† $p < 0.01$.
Ant. = anterior and Post. = posterior.

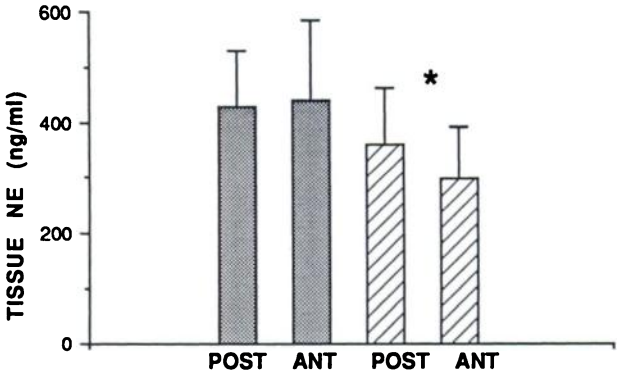

FIGURE 3
Tissue norepinephrine concentration in anterior (ant) and posterior (post) segments of left ventricle in control animals (dark bars) and intervention animals (hatched bars) * = $p < 0.05$.

inhibition) caused reduction of myocardial ^{18}F activity 30 min following i.v. injection by 82% and 74%, respectively (*23*). These experimental data indicate the specificity of this tracer for the norepinephrine uptake in nerve terminals and suggest little non-neuronal uptake. Previous studies in our laboratory have demonstrated a close correlation between tissue norepinephrine content and retention of FMR in a dog model of regional myocardial denervation (*22*).

Kinetic data using intracoronary injection of the tracer demonstrate a bioexponential clearance from myocardium (*24*). There was a high first-pass extraction of FMR (70%–80%) followed by a rapid clearance of unbound activity from myocardium. The retained activity averaged ~ 70% of peak myocardial activity and remained stable with a long biologic tissue half-life, explained by the fact that FMR is not metabolized in neurons and equilibrates with the large neuronal norepinephrine pool. In the current study, tissue activity was measured 30 min after i.v. tracer injection. Therefore, no information was derived about the initial uptake of this tracer and clearance kinetics in the normal and reperfused myocardium. It is assumed that the ^{18}F activity at 30 min represents equilibration of the tracer in the tissue norepinephrine stores, while most of the non-neuronally bound FMR has cleared from tissue at this time. Thus, the ^{18}F activity at 30 min reflects predominantly the catecholamine storage capacity of sympathetic nerve terminals. This assumption is supported by agreement of the relative reduction of tissue norepinephrine content and FMR retention in the reperfused myocardium. However, it is difficult to differentiate between increased release of FMR and decreased initial uptake of this tracer as cause for the regional reduction of FMR retention. On the other hand, the regional alterations observed in reperfused myocardium are likely to reflect ischemically induced changes of uptake and storage, since increased release by central nervous stimulation would affect both the normal and reperfused myocardium. Ongoing reserach in our institution employing dynamic positron emission tomography addresses the FMR kinetics in ischemic and postischemic myocardium to further characterize the regional uptake and clearance of this tracer from ischemically injured myocardium. The tracer kinetic model for FMR is being currently validated and may in the future permit the quantification of uptake and release of neurotransmitters from sympathetic nerve terminals (*24*).

TABLE 3
Regional FMR Uptake in Control Animals (Group 1)

	Ant.*	Post.†
1	30	30
2	26	30
3	30	32
4	33	37
5	29	31
6	22	23
	28.3 ± 3.8	30.5 ± 4.5

* Ant. = anterior wall supplied by LAD artery. This study was isolated and a suture looped around it.
† Post. = posterior wall.

Data Interpretation

Previous investigations have not shown depletion of tissue norepinephrine content after 1–3 hr of coronary artery occlusion, but a redistribution of norepinephrine from the neuronal cells into the extracellular space (*27*). Histochemical studies showed significant reduction of catecholamine containing sympathetic nerve terminals in the presence of unchanged tissue norepinephrine content. Upon restoration of blood flow norepinephrine is released from ischemic myocardium as evidenced by increased norepinephrine spillover determined by arterio-venous (coronary sinus) blood sampling (*19,28*). This washout of tissue catecholamines is followed by prolonged depletion of tissue norepinephrine content following ischemia (*29*). Accordingly, myocardial de-

nervation has been observed distally to the site of tissue necrosis presumably due to neuronal damage of proximal nerve fibers supplying the myocardium distally to the ischemic injury (*11*).

The new tracer approach employed in this investigation indicates sustained functional impairment of sympathetic nerve terminals following relatively short duration of ischemia. Thirty minutes of coronary occlusion have been chosen to produce an ischemic injury without significant tissue necrosis (*30*). The observed results suggest decreased norepinephrine uptake and storage in injured, but viable, myocardium. Norepinephrine is stored together with high energy phosphates in the nerve terminals (*31*). This energy dependent process has been shown to be sensitive to ischemia and acidosis (*34,35*). The impairment of vesicular storage of norepinephrine leads to increased catecholamine concentration in the neuronal cytosol (*36*). Following shorter time periods of ischemia (15–40 min, isolated rat heart), the release of norepinephrine has been linked to a concentration gradient driven reversal of the uptake I mechanism on the neuronal cell membrane. Such underlying pathophysiology is in agreement with our observation of decreased neuronal retention of the norepinephrine analogue FMR. The reduced tracer retention 30 min following injection may reflect the decreased neuronal norepinephrine pool and reduced inward transport of norepinephrine into the neuron. The altered norepinephrine uptake mechanism may explain the greater reduction of FMR retention as compared to tissue norepinephrine reduction. Thus, the tracer approach may provide increased sensitivity to define the functional impairment of the sympathetic nerve terminal. The definition of tracer kinetics by dynamic imaging protocols is expected to quantitatively assess the uptake and retention of FMR, yielding further elucidation of the ischemically induced alterations of neuronal function (*24*).

Sympathetic neurons travel the surface of the heart along the vascular structures. To assess the effect of surgical manipulation of the LAD artery on the distal nerve function, a sham animal group was included. In these animals, a snare was placed around the LAD, but the vessel was not occluded. The tissue norepinephrine content determined post mortem in the anterior wall was not different as compared to the posterior wall. However, the FMR retention was slightly, but significantly reduced (−7%) in the anterior wall (Table 3).

Animals studies have shown that norepinephrine depletion following proximal nerve dissection requires several days (*37*). However, the reuptake and storage of catecholamine may be more sensitive to nerve injury and precede norepinephrine depletion. The observed small changes in FMR retention indicate the sensitivity of the tracer approach in assessing neuronal integrity and suggest that even minor manipulation of the coronary arteries may cause alterations in distal neuronal function. This finding may be of importance for animal studies employing open chest models with dissection of coronary arteries. The magnitude of decreased FMR retention, however, was significantly less than in the intervention group indicating a direct ischemic effect on FMR retention beyond the expected changes due to the surgical instrumentation.

CONCLUSION

The decreased FMR retention in reperfused myocardium following short duration of ischemia demonstrate the sensitivity of the sympathetic nervous system to transient ischemia. The tracer approach with FMR appears promising for the noninvasive delineation of the sympathetic nervous system of the heart in vivo. Future imaging studies with dynamic data acquisition are required to elucidate the tracer kinetics in ischemically injured myocardium and to define the time course of neuronal recovery following ischemic insults. The observations of this study may be of importance in patients with unstable angina and myocardial infarction. Severe ischemia may cause regional "denervation" of ischemically injured but viable myocardium. Such heterogeneity in neuronal function may produce regionally increased extra-cellular norepinephrine concentration, which may alter electrophysiologic behavior and facilitate dysrhythmia.

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