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MYOCARDIAL OXYGENATION IN DOGS DURING REACTIVE HYPEREMIA

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ABSTRACT

The mechanisms of myocardial oxygenation during reactive hyperemia were studied in the beating heart using continuous near infrared (NIR) spectroscopy. In open chest dogs, NIR spectroscopy was used to monitor brief occlusions of the left anterior descending artery. These occlusions produced a precipitous drop in tissue oxygen stores ($t\mathrm{HbO_2}$+$\mathrm{MbO_2}$), tissue blood volume, and the oxidation level of mitochondrial cytochrome a,a_3. Reperfusion produced a rapid increase in the NIR signals to supranormal levels, followed by gradual return to baseline. When the duration of occlusion was increased from 20 to 120 s, an essentially linear increase was produced in the overshoot areas defined by the NIR signals. Near infrared spectroscopy (NIRS) separated reactive hyperemia into two phases according to the tissue level of deoxyhemoglobin and deoxymyoglobin (tHb+Mb): (1) an early phase during which the tHb+Mb level was supranormal, reflecting enhanced O_2 extraction; and (2) a late phase during which the tHb+Mb level was below baseline, reflecting decreased O_2 extraction and increased tissue O_2 availability. During reactive hyperemia, when O_2 availability was maximal by NIR spectroscopy, O_2 consumption was elevated but submaximal, indicating that MVO_2 was not limited by O_2 availability. Cytochrome a,a_3 oxidation state also was restored fully. Thus, myocardial oxygenation is highly regulated during reactive hyperemia. Cellular O_2 supply and mitochondrial oxidation state are restored early during reactive hyperemia by increased O_2 delivery, increases in tissue blood volume and enhanced O_2 extraction. © *1998 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(98)00502-4]

Keywords cytochrome oxidase; myocardial oxygenation; myocardial oxygen consumption; reactive hyperemia; adenosine; near infrared spectroscopy; radiolabeled microspheres.

1 INTRODUCTION

Reperfusion after coronary artery occlusion transiently increases myocardial blood flow to supranormal levels. This response, termed ''reactive hyperemia'' is also accompanied by a transient increase in oxygen consumption and contractile activity to supranormal levels.[1–3] Increasing the duration of ischemia increases in the magnitude of hyperemia as well as the oxygen consumption, suggesting a highly regulated recovery from the crisis in myocardial energy provision.[4,5,1] Myocardial respiration during reperfusion does not appear to be limited by blood flow (or oxygen delivery) since reactive hyperemia is associated with a transient decrease in tissue oxygen extraction to below control levels.[4,3] The mechanisms controlling myocardial respiration during reactive hyperemia remain incompletely understood.[6]

Transmural changes in myocardial oxygen availability *in vivo* can be assessed continuously using near infrared (NIR) spectroscopy.[7–9] This nondestructive approach permits rapid, concommitant measurement of changes in tissue blood volume and oxygenation of tissue hemoglobin and myoglobin. In addition, information about the oxidation-reduction state of mitochondrial cytochrome a,a_3 can be obtained by monitoring changes in the oxidation state of the copper center (Cu_A). However, this signal is weak and subject to error compared to hemoglobin.

In the present study, qualitative NIR optical responses were monitored along with regional myocardial oxygen delivery and oxygen consumption during (1) reactive hyperemia and (2) adenosine-induced hyperemia in the beating canine heart. We hypothesized that the mitochondrial oxidation state is regulated at near normal levels after brief ischemia despite excessive oxygen availability during reactive hyperemia. We found fundamental differ-

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ences in tissue oxygen availability under the two study conditions which suggest preservation of normal relationships between myocardial oxygenation and reactive hyperemia after brief ischemia.

2 METHODS

2.1 PREPARATION OF ANIMALS

The experimental preparation has been described previously, with modifications as indicated below.[7,8] Adult mongrel dogs weighing 25–35 kg were anesthetized with intramuscular morphine sulfate 1.5 mg/kg, and α chloralose (160 mg/kg over 10 min followed by 10 mg/kg/hr intravenously), endotracheally intubated, and ventilated on room air. Arterial blood gases were acquired periodically to ensure adequate systemic oxygenation and acid-base balance. The heart was exposed via a left thoracotomy at the sixth intercostal space, and a pericardial cradle was formed. Heparin-filled polyvinyl chloride catheters (3 mmOD) were inserted in the carotid artery for systemic blood pressure measurements and withdrawal of microsphere reference samples, and in the left atrium for microsphere injections. A segment of the left anterior descending coronary artery of approximately 1.0 cm in length was isolated proximal to the first diagonal branch and a pneumatic cuff occlude was positioned around the vessel to allow total occlusion. A Howell-ST electromagnetic flow probe, previously calibrated using an *in vitro* system, was positioned just proximal to the pneumatic cuff occluder. Two separate fiberoptic bundles (optodes), one to deliver near infrared light and one to receive reflected light, were positioned 2 cm apart on the epicardial surface within the region to be rendered ischemic. In some experiments, a heparinized 24 gauge catheter was placed in the great cardiac vein to acquire samples of venous effluent from the region of illumination. Lead II of the electrocardiogram, systemic blood pressure, and phasic coronary blood flow were recorded on a Gould Model 412 oscillograph. Near infrared data were recorded simultaneously using a computer and printer. The signals were synchronized using a marker connected to the two recorders.

2.2 EXPERIMENTAL PROTOCOLS

Approximately 30 min were allowed for stabilization of the preparation, during and after which the above parameters were recorded continuously. To assess the effect of the duration of ischemia on myocardial oxygenation during reactive hyperemia, one group of dogs (group A) underwent total left anterior descending (LAD) coronary artery occlusion for 20–120 s, in 20 s increments. The recovery period between occlusions was 10–20 min, depending on the duration of the occlusion. Radiolabeled microspheres were injected into the left atrium and arterial blood samples were collected for measurement of tissue blood flow prior to ischemia (baseline), during the 120 s occlusion (beginning 30 s after occlusion), and during hyperemia (beginning 30 s after release of occlusion). Sampling of arterial blood required ~80 s.

To assess myocardial oxygenation during reactive hyperemia and during adenosine-mediated hyperemia, a different protocol was employed in another group of dogs (group B). Radiolabeled microspheres were injected into the left atrium and arterial blood samples were collected for measurement of tissue blood flow and O_2 content under basal conditions. Mixed venous blood was withdrawn simultaneously from the great cardiac vein at a rate of 0.1 ml/s, collected in a heparinized syringe and placed on ice for immediate measurements. Hemoglobin saturation and concentration were measured on a Co-oximeter (IL Model 413) using a canine algorithm to determine the O_2 content of venous and arterial blood samples. LAD coronary artery flow was reduced progressively by 5%–19% at 20 s intervals by graded inflation of the pneumatic snare as NIR optical responses were monitored.[8] When the cytochrome a,a_3 oxidation level had decreased by 20%–40%, the pneumatic snare was clamped to maintain inflation, and radiolabeled microspheres again were injected. Blood samples were acquired, as above, for microsphere reference levels and for arterial and venous O_2 content determinations. The coronary occlude was released ~100 s after the second microsphere injection for a recovery period of ~20 min. The LAD coronary artery then was abruptly and completely occluded for 2 min. At 30 s into occlusion, microspheres were injected for a third time, and blood samples again were acquired for microsphere reference and for arterial and venous O_2 content measurements. The coronary snare was then released, and 30 s into reperfusion, when the cytochrome a,a_3 oxidation level was maximal, a fourth set of tissue blood flow and O_2 content measurements were acquired. Following a recovery period of 20 min, adenosine was infused intravenously at a rate of 0.5 mg/kg/min. After the NIR optical responses had stabilized (~10 min), tissue blood flow and O_2 content measurements were acquired for a fifth time. The adenosine infusion rate was then increased to 1.0 mg/kg/min, and, after an equilibration period of ~10 min, a final set of tissue blood flow and O_2 content measurements were acquired.

2.3 *IN VIVO* OPTICAL MONITORING

We used a method for NIR spectroscopy in the *in situ* canine heart which has been published previously.[7,8] This method is used only for trend monitoring of changes in absorption of the oxidized copper moiety (Cu_A) of cytochrome a,a_3, and the iron-porphyrin centers of oxygenated and deoxygenated hemoglobin and myoglobin ($t\mathrm{HbO_2}$+$\mathrm{MbO_2}$,tHb+Mb). The sum of the $t\mathrm{HbO_2}$

and tHb+Mb signals cancels the effects of changes in myoglobin saturation, permitting the measurement of changes in tissue blood (hemoglobin) volume (tBV).

Near infrared spectroscopy exploits the ready propagation of light through tissue (photon migration) in the 700–900 nm wavelength range.[10] The composite absorption data from the overlapping spectra are deconvoluted by multiwavelength algorithms, derived under the actual absorption and light scattering conditions present in living tissue.[8,11,12] The algorithms used in this study were scaled to provide signal amplitudes of approximately equal size for the HbO_2, Hb, and Cu_A, and in this form, cannot be used to compare the concentrations of Cu_A with those of HbO_2 or Hb.

The NIR spectrometer delivered pulsed light at four different wavelengths (775, 810, 870, and 904 nm) to the tissue via the fiberoptic optodes. Reflected light was collected by a second optical bundle, after which the photosignals were processed into metabolic signals as described previously.[13,7,8,11,14] The geometry was kept constant during an experiment by immobilizing the optodes with a stereotaxic device. Metabolic trends were monitored after a baseline is established for each optical parameter under well-defined normal physiologic conditions.

Optical responses associated with ischemia and reperfusion have been expressed as changes in relative optical density ($\triangle$OD). For the derived signals for Cu_A, tBV, tHbO_2+MbO_2 and tHb+Mb, the data have been expressed as the total labile signal, or the maximal dynamic range during each experiment.[8] For hemoglobin and myoglobin, the molar occupancy of heme moieties by oxygen is related linearly to changes in the concentration of tissue oxyhemoglobin plus oxymyoglobin (tHbO_2+MbO_2).[9] The latter quantity has been termed the ''tissue oxygen store'' because it accounts for essentially all of the oxygen bound to tissue hemoglobin and myoglobin.[13,7,8,3] Changes in these signals relate linearly to changes in concentration, though absolute concentration could not be determined by the present method, since values for optical pathlength in the heart are unknown.[15] The tissue oxygen store reflects the influx of oxyhemoglobin into the tissue region of illumination via small arteries and arterioles, the uptake of O_2 from hemoglobin and myoglobin by mitochondria, and the efflux of remaining oxyhemoglobin via venous channels. Changes in the oxyhemoglobin component of tissue O_2 stores primarily reflect changes in the capillary and venous O_2 content, since most of the hemoglobin is located in the capacitance vessels of the heart, and since arterial O_2 content is relatively constant during ischemia or hyperemia.[16,1,17,8] Similarly, changes in the deoxyhemoglobin component of the tHb+Mb response relate primarily to changes in the volume of deoxyhemoglobin in the venous blood. Under normoxic conditions (SaO_2~95%), if the mixed venous O_2 saturation is ~35% and ~75% of the blood volume is located in the capacitance vessels, the mean tissue hemoglobin is $(0.95+0.35\times3)/4$ or ~50%. Accordingly, ~50% of the hemoglobin within the region of illumination is oxyhemoglobin and ~50% is deoxyhemoglobin. If myoglobin operates near its P_{50}, about half of the total heme signal in the beating dog heart under control conditions is comprised of tHbO_2+MbO_2 and the remainder is tHb+Mb.[18] Perturbations that produce a change in mean tissue heme saturation (hemoglobin and/or myoglobin) are reflected in a directionally similar change in the tHbO_2+MbO_2 level and a reciprocal change in the tHb+Mb level.

2.4 QUANTITATION OF MYOCARDIAL BLOOD FLOW

Regional myocardial blood flow was measured using 11.4 ± 0.01 μm radiolabeled microspheres as described.[19,7,8] After the experiments, the locations of the optodes on the epicardium were marked with pins, and the hearts were removed and fixed in 10% buffered formalin. Two contiguous 1 cm^2 full-thickness sections of myocardium were excised from the tissue region spanning the sites of photon entry and photon reception. Each section was sliced into four layers of equal thickness from epicardium to endocardium. The remainder of the left ventricle was sectioned similarly. All sections were weighed and counted in a gamma spectrophotometer (Canberra Series 35-Plus). Blood flow was calculated and expressed in ml/min/gm wet weight. The product of mean transmural blood flow, at the site of illumination, and the arterial-minus-venous O_2 content difference was used to quantitate myocardial oxygen consumption by the Fick equation.[3]

2.5 DATA ANALYSIS

Summed data were expressed as mean ± standard deviation, unless otherwise stated. Differences in paired data were determined by paired t test. To account for multiple comparisons, p values were adjusted according to the Bonferonni inequality. A p value of <0.05 was considered statistically significant. To quantitate the changes in the NIR signals during reactive hyperemia (group A), the change in optical density ($\triangle$OD) of each absorber (tHbO_2+MbO_2, tHb+Mb, cytochrome a,a_3) was integrated over time (seconds) using a Digi-Pad 5 (GTCO Co.) and was expressed in $\triangle$OD● second units. The integrated areas also were measured by an independent observer to confirm that they did not differ significantly for any of the optical parameters.

3 RESULTS

The effect of the duration of ischemia on myocardial oxygenation during reactive hyperemia was as-

sessed in the dogs in group A. Complete studies were obtained in eight animals. Baseline hemodynamic values included a heart rate of 112 ±30 beats/min, an arterial systolic blood pressure of 127±9 mmHg, and a diastolic blood pressure of 88±17 mmHg.

Baseline transmural blood flow at the site of the optodes, assessed by radiolabeled microspheres, averaged 0.77±.21 ml/min/gm. During LAD coronary artery occlusion, mean transmural flow decreased to 0.19±16 ml/min/gm ($p<.001$ versus baseline levels). Upon reperfusion after 120 s of ischemia, the mean flow increased to 2.28 ±.67 ml/min/gm ($p<.001$ compared to baseline and ischemic flows).

3.1 NIR OPTICAL RESPONSES DURING ISCHEMIA AND REPERFUSION

After onset of coronary artery occlusion, the oxidation level of Cu_A decreased almost immediately, due to an imbalance between electron flux and oxygen supply. Upon reperfusion, the oxidation level of the enzyme increased to ~15% above preocclusion levels followed by rapid normalization, as shown previously.[7] The overshoot in cytochrome a,a_3 oxidation level during reperfusion defined an area (area "*a*" in Figure 1) which increased as a near linear function of the duration of ischemia, for occlusions of 20–120 s in duration [Figure 2(a)].

Tissue blood volume decreased abruptly upon coronary artery occlusion, and increased to supranormal levels during reperfusion, followed by gradual normalization. The *t*BV signal, however, became maximal sooner and normalized earlier in reperfusion than did the cytochrome oxidation level.[7] The area defined by the *t*BV overshoot (area "*b*" in Figure 1) increased linearly as a function of the duration of ischemia [Figure 2(a)].

The tissue level of oxyhemoglobin and oxymyoglobin ($t\text{HbO}_2$+MbO_2) decreased rapidly during coronary occlusion, and increased to supranormal levels during reperfusion in concert with the cytochrome oxidation level. This signal returned to preocclusion baseline more gradually than either the *t*BV or the cytochrome optical responses.[7] Like the *t*BV and cytochrome oxidation levels, the area defined by the overshoot in the $t\text{HbO}_2$+MbO_2 level during reactive hyperemia (area "*c*" in Figure 1) increased linearly as a function of the duration of ischemia [Figure 2(b)].

The change in the tissue level of deoxyhemoglobin and deoxymyoglobin (*t*Hb+Mb) associated with coronary occlusion and reperfusion is also shown in Figure 1. Unlike the other NIR optical responses, the pattern of the *t*Hb+Mb response during reperfusion consisted of an initial increase followed by a decrease to below baseline, and then by gradual return to baseline.[7] The area defined by the initial increase in *t*Hb+Mb (overshoot area "*d*" in

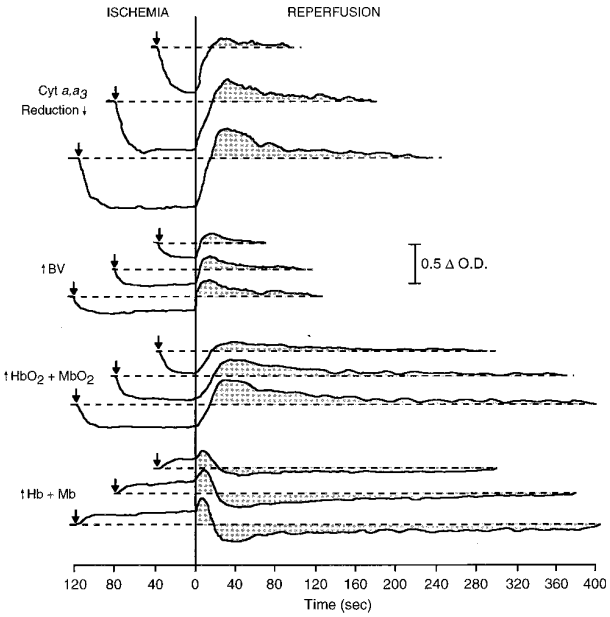

Fig. 1 Near infrared optical responses in canine myocardium during 40, 80, and 120 s LAD artery occlusions and reperfusion. Representative recording from one of eight dogs. TOP—The cytochrome a,a_3 oxidation level during reperfusion delineates an overshoot area (a) which increases with increasing duration of occlusion. UPPER MIDDLE—The *t*BV response during reperfusion defines an overshoot area (b) which increases with increasing duration of occlusion. LOWER MIDDLE—The $t\text{HbO}_2$+MbO_2 responses during reperfusion delineates the O_2 store overshoot area (c) which increases with increasing duration of occlusion. BOTTOM—The *t*Hb+Mb response during reperfusion defines an early overshoot area (d) and a late undershoot area (e). Both areas increase with increasing duration of occlusion. Arrow indicates onset of coronary occlusion. Solid vertical line indicates release of coronary occlusion. Cyt a,a_3, cytochrome a,a_3; *t*BV, tissue blood (hemoglobin) volume; $t\text{HbO}_2$+MbO_2, tissue oxyhemoglobin plus oxymyoglobin; *t*Hb+Mb, tissue deoxyhemoglobin plus deoxymyoglobin; △OD, change in optical density.

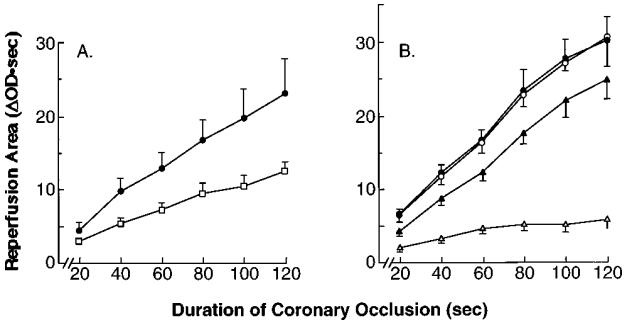

Fig. 2 Overshoot areas defined by NIR optical responses following LAD occlusion of 20–120 s in canine myocardium. Data are presented as mean ±S.E.M. for eight dogs. (a) Cytochrome a,a_3 overshoot area (closed squares) and tissue blood volume overshoot area (open squares) increase with increasing duration of occlusion. (b) $t\text{HbO}_2$+MbO_2 (O_2 store) overshoot area (open circles), *t*Hb+Mb undershoot area (closed triangles), *t*Hb+Mb overshoot plus undershoot area (close circles), and *t*Hb+Mb overshoot area (open triangles) increase with increasing duration of occlusion.

Table 1 Myocardial blood flow, O_2 extraction, O_2 delivery, and O_2 consumption during coronary occlusion and reperfusion.

	BASAL	Partial occlusion	Complete occlusion	Reperfusion
Transmural blood flow (ml/min/gm)	0.84±.23	0.41±.15*	0.23±.13*†	2.06±.94*
CaO_2 (ml O_2/dl)	18.8±2.4	18.9±2.6	19.1±2.5	18.8±2.4
CvO_2 (ml O_2/dl)	9.8±2.1	6.7±1.9*	8.0±2.2*†	11.9±2.7
CaO_2–CvO_2 (ml O_2/dl)	9.0±2.3	12.2±3.2*	11.1±2.5*	7.1±2.0
O_2 delivery (ml O_2/min/100 gm)	15.8±5.1	7.7±2.7*	4.5±2.4*†	39.0±15.6*
MVO_2 (ml O_2/min/100 gm)	7.5±2.7	4.8±1.8*	2.5±1.1*†	14.0±5.6*

Values are mean±S.D. for nine dogs.

* $p<.05$ vs BASAL (preocclusion) levels.

† $p<.05$ vs partial occlusion.

Abbreviations: CaO_2=arterial O_2 content; CvO_2=mixed venous O_2 content from great cardiac vein; MVO_2=myocardial O_2 consumption.

Figure 1) increased linearly with increasing duration of occlusion up to ~60 s, and increased more gradually for occlusions of 80 to 120 s [Figure 2(b)]. The area defined by the decrease in tHb+Mb below baseline (undershoot area "*e*" in Figure 1) increased linearly with increasing duration of occlusion [Figure 2(b)]. Also as shown in Figure 2(b), the sum of the tHb+Mb overshoot and undershoot areas was approximately equal to the $t\mathrm{HbO_2}+m\mathrm{BO_2}$ overshoot area.

3.2 O_2 AVAILABILITY VERSUS MVO_2 DURING ISCHEMIA AND REPERFUSION

To further characterize myocardial oxygenation during reactive hyperemia, the oxidation level of cytochrome a,a_3 (Cu_A) was assessed in relation to myocardial oxygen consumption during ischemia and reperfusion in the dogs in group B. Complete studies were obtained in nine animals. Baseline hemodynamic values were similar to group A, including a heart rate of 110±31 beats/min, an arterial systolic blood pressure of 125±24 mmHg, and a diastolic blood pressure of 94±14 mmHg.

As shown in Table 1, partial coronary occlusion produced a ~51% decrease in transmural blood flow and a ~36% decrease in MVO_2, while complete occlusion produced a ~73% decrease in blood flow and a ~66% decrease in MVO_2. At ~30 s into reperfusion, there was a 145% increase in tissue blood flow and an 87% increase in MVO_2 above control levels. Although the MVO_2 and Cu_A oxidation state measurements are subject to errors related to differences in sampling volume, we correlated the *in vivo* relationship of MVO_2 to Cu_A oxidation state using to a polynomial equation. The best fit curve for these qualitative measurements is shown in Figure 3. Cytochrome a,a_3 was partially reduced (~85% oxidized) in the beating canine heart under control conditions.[7] In ischemia the relationship between the Cu_A oxidation level and MVO_2 was essentially linear, suggesting that myocardial respiration was limited by O_2 availability under these conditions.[8] Since both the Cu_A oxidation level and the $t\mathrm{HbO_2}$+$\mathrm{MbO_2}$ level became maximal at ~30 s into reperfusion (Figures 1 and 3, Table 1), this likely represents the point of maximal myocardial O_2 availability during reactive hyperemia. As indicated above, MVO_2 was only increased by 87% above control levels when myocardial O_2 availability was maximal by NIRS (Figure 3, Table 1). Figure 3 also shows the relationship of the NIRS signal for the O_2 store and Cu_A during the ischemia experiments (inset). The apparent one-to-one relationship between $t\mathrm{HbO_2}$+$\mathrm{MbO_2}$ and Cu_A can best be explained if partial and complete ischemia cause drop out of entire perfusion-metabolism units, e.g., entire groups of myocytes are lost during ischemia wherein both the mitochondrial oxidase and the hemoglobin (+ myoglobin) become reduced and deoxygenated simultaneously.

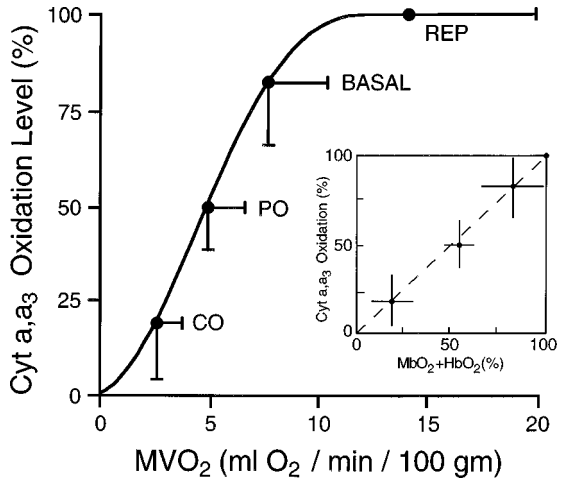

Fig. 3 Relationship of cytochrome a,a_3 oxidation level to myocardial oxygen consumption (MVO_2) prior to coronary occlusion (BASAL), during partial occlusion (PO), during complete occlusion (CO), and at 30 s into reperfusion (REP) in canine myocardium. Cytochrome oxidation level is expressed as % of total labile signal (see Methods). Data are presented as mean ±S.D.> for nine dogs. Relationship was best fit using MacIntosh software by a fourth-order polynomial equation: $Y=1.008X+3.574X^2-0.387X^3+0.012X^4$ ($r^2=1.00$), the curve for which is shown.

3.3 MYOCARDIAL OXYGENATION DURING ADENOSINE-MEDIATED HYPEREMIA

Myocardial oxygenation during reactive hyperemia was compared to during pharmacologic vasodilation with adenosine in the same animals. Complete studies were obtained in seven of the nine dogs in group B. As shown in Table 2, the baseline parameters of myocardial perfusion and oxygenation in this subgroup were similar to that of the entire group (Table 1). During infusion of adenosine at 0.5 mg/kg/min, tissue blood flow increased by 82% above control levels. Despite the increase in arterial O_2 content, the $(A\text{-}V)O_2$ content difference decreased such that MVO_2 did not change significantly. NIR spectroscopy detected increases from control in (1) the tissue blood volume, reflecting adenosine-mediated vasodilation, and (2) the tissue level of oxyhemoglobin and oxymyoglobin ($t\text{HbO}_2+\text{MbO}_2$), reflecting the decrease in O_2 extraction (Figure 4). Both changes were statistically significant during infusion of adenosine at 1.0 mg/kg/min. There was not a statistically significant change in the tissue level of deoxyhemoglobin and deoxymyoglobin (tHb+Mb) during infusion. Also, the oxidation level of Cu_A did not change during infusion, despite the adenosine-mediated increase in myocardial O_2 delivery.

4 DISCUSSION

The dynamic changes in myocardial oxygenation during reactive hyperemia have been difficult to study due to a lack of rapid, continuous techniques to monitor the events. Using NIR spectroscopy to study reactive hyperemia, we found a linear relationship between the duration of coronary occlusion and the magnitude of change in the tissue blood volume, $t\text{HbO}_2+\text{MbO}_2$, and tHb+Mb levels (Figures 1–3). Also, the Cu_A signal generated through our algorithms followed the $t\text{HbO}_2+\text{MbO}_2$ signals (see below). This relationship was apparent

Table 2 Myocardial blood flow, O_2 extraction, O_2 delivery, and O_2 consumption during adenosine infusion.

	BASAL	Adenosine 0.5 mg/kg/min	Adenosine 1.0 mg/kg/min
Transmural blood flow (ml/min/gm)	0.88±.24	1.60±.46*	1.48±.75
CaO_2 (ml/O_2/dl)	19.2±2.4	21.0±2.2*	21.9±2.6*
CvO_2 (ml/O_2/dl)	9.9±1.4	17.0±2.2*	17.6±1.6*
CaO_2–CvO_2 (ml O_2/dl)	9.3±2.3	4.0±1.4*	4.3±1.7*
O_2 delivery (ml O_2/min/100 gm)	16.9±5.3	34.9±11.6*	33.1±18.5*
MVO_2 (ml O_2/min/100 gm)	8.1±2.7	6.7±3.2	6.2±3.1

Values are mean ±S.D. for seven dogs.

* $p<.05$ vs BASAL levels.

Abbreviations: CaO_2=arterial O_2 content; CvO_2=mixed venous O_2 content from great cardiac vein; MVO_2=myocardial O_2 consumption.

over a sixfold range in occlusion duration, implying that myocardial oxidative metabolism is highly regulated following coronary occlusions of up to two minutes in duration.

When interpreting the NIRS signals in this study, it should be remembered that some confusion exists in the literature concerning the *in vivo* oxidation-reduction behavior of the Cu_A center of cytochrome oxidase. Part of this confusion relates to the methods used to develop algorithms, e.g., *in vivo* versus *in vitro*, and part relates to the actual biochemical behavior of Cu_A *in vivo* in the presence of hemoglobin which contributes nearly 90% of the total NIR optical signal in the heart. These problems have produced some debate about the validity of the Cu_A signal and whether Cu_A undergoes a reduction response during ischemia. It is important to note that the algorithms used in this study are based on *in vivo* spectra obtained in the absence of hemoglobin when the oxidized NIR absorption band of Cu_A was demonstrated to disappear during ischemia.

The Cu_A and hemoglobin algorithms in this study are scaled to provide trend signals of about the same amplitude. These algorithms cannot be used to compare concentration changes for hemoglobin and Cu_A (Ref. 20) because they are designed to provide signals of approximately the same magnitude full scale and not changes in absolute concentration. *In vivo*, the actual total contribution of the Cu_A absorption is only about one-eighth of the total for HbO_2+MbO_2. Such trend monitoring algorithms can be modified to produce signals appropriate for comparing actual concentrations of Cu_A and hemoglobin to each other, however, other critical factors such as optical pathlength are not known for the intact heart.

The NIR deoxyhemoglobin plus deoxymyoglobin (tHb+Mb) signal during reperfusion defined two distinct phases of reactive hyperemia, which were not discernable by analysis of the $t$$HbO_2$+$MbO_2$ or Cu_A optical responses (Figure 1). During the early phase, defined by the tHb+Mb overshoot (area ''*d*'' in Figure 1), the oxidation level of Cu_A was below preischemic baseline, reflecting limited mitochondrial oxygen availability (Figure 1). This initial phase of reactive hyperemia was characterized by increased cellular extraction of oxygen from hemoglobin as it entered the ischemic tissue, resulting in increased tHb+Mb levels.[21,22,7] Earlier studies indicate that a rapid and transient increase in MVO_2 to 200%–300% above preischemic levels occurs when the level of deoxyhemoglobin in the venous effluent is maximal, less than 10 s into reperfusion, and MVO_2 remains markedly elevated for only the initial ~20 s of reactive hyperemia.[4,3] Within this brief period, which corresponds to the period of increased tHb+Mb in the present study, high energy phosphates are replenished, suggesting that bioenergetic recovery after brief ischemia occurs early during reactive hyperemia.[6] The magnitude of the MVO_2 overshoot related to the duration of ischemia, implying that metabolic impairment during ischemia is linked to metabolic recovery during reperfusion.[4,3]

Since maximal hyperemia and O_2 delivery occurs after coronary occlusions of only 20 s in duration,[23] the additional O_2 demand produced by longer occlusions was met by increasing O_2 extraction.[23,7] Accordingly, the magnitude of the tHb+Mb overshoot area increased as the duration of ischemia was increased from 20 to 120 s [Figures 1 and 2(b)]. The increase in tHb+Mb was not linear as a function of occlusion duration [Figure 2(b)], presumably because MVO_2 (and O_2 demand) decline toward a nadir level defined by the availability of collateral blood flow as the duration of coronary occlusion was increased from 20 to 120 s.[7,8] Thus, the increase in the tHb+Mb level upon entry of oxygenated blood into the microcirculation likely reflects oxidative recovery after brief ischemia. The factors involved are (1) diffusion of O_2 down a steep gradient from the intravascular to the intracellular compartment to normalize mitochondrial pO_2 and replenish myoglobin O_2 stores, and (2) a compensatory increase in O_2 extraction from hemoglobin to accommodate an increase in cellular O_2 demand, the magnitude of which relates to the duration of coronary artery occlusion.

A second phase of reperfusion, which was defined by the tHb+Mb undershoot (area ''*e*'' in Figure 1), occurred during the remainder of hyperemia.[7] During this later phase, tissue pO_2 is elevated[24,25] and hemoglobin saturation of the venous effluent is increased (Table 1) (Refs. 4 and 3). The NIR method indicates the tissue level of oxyhemoglobin plus oxymyoglobin ($t$$HbO_2$+$MbO_2$) is supranormal at this time (Figure 1), suggesting increased tissue oxygen availability. Increased mitochondrial O_2 availability is also suggested by the concomitant increase in the Cu_A oxidation level to above preocclusion levels, though decreases in NADH or ADP availability could also influence the redox state of the terminal oxidase.[7,26–28] The increase in oxygen availability during this period has been postulated to be due to the combined effects of sustained hyperemia (and O_2 delivery) and rapidly declining tissue oxygen demand.[3]

Tissue blood volume increased above baseline levels during reperfusion (Figure 1), indicating that the intravascular space expanded transiently following ischemia.[7] Since the magnitude of the tBV overshoot increased linearly as a function of the duration of coronary occlusion (Figure 2), tissue blood volume is clearly regulated in response to myocardial ischemia. The increase in tissue blood volume may reflect capillary recruitment to enhance oxygen diffusion early during reactive hyperemia when O_2 availability is limited.[29] In addition to expediting reoxygenation, this vasodilatory response may en-

hance the efficiency by which metabolites are washed out during reperfusion. The mechanism(s) regulating the overshoot in tissue blood volume are largely unknown. While this response may be mediated in part by endogenous vasodilators, adenosine is an unlikely candidate since it does not influence the initial increase in blood flow or maximal hyperemia which occur when tissue blood volume is maximal.[30,23,7,31]

To further investigate myocardial oxygenation during reactive hyperemia, we assessed mitochondrial oxygen availability and MVO_2 within the same region of the beating dog heart during ischemia and reperfusion. In ischemia, oxygen consumption declines because oxygen becomes limiting to populations of myocytes (decreased $t\mathrm{HbO_2}$+$\mathrm{MbO_2}$) and hence, to their mitochondria.[32] If the flow of electrons ceases at cytochrome a,a_3, and the enzyme stops consuming oxygen, and the oxidation state of Cu_A decreases. The linear relationship shown in Figure 3 between MVO_2 and the Cu_A oxidation state when tissue blood flow was restricted suggests critical dependence of myocardial respiration on oxygen availability when oxygen delivery becomes limited.[7,8] If oxygen availability limited the MVO_2 during reperfusion, as it does during ischemia, the maximal (200%–300%) increase in MVO_2 would be expected to occur when the Cu_A oxidation level was maximal.[4,3] Our data indicate, however, that MVO_2 was only 87% above preischemic levels when the Cu_A oxidation level became maximal 30 s into reperfusion (Table 1 and Figure 3). Thus, myocardial respiration does not appear to be limited by oxygen availability during reperfusion. This point is supported by the observation described above that maximal O_2 consumption occurs early during reactive hyperemia when O_2 availability is below control levels, rather than later in hyperemia when O_2 availability is supranormal.[4,3] The precise factors regulating the rate of aerobic respiration during reperfusion cannot be defined from the present data, though limitations of NADH or ADP may be involved.[26,33]

The mechanisms relating hyperemia to myocardial oxygenation were further studied by comparing tissue oxygenation during reactive hyperemia with pharmacologic vasodilation by adenosine in the same animals. Adenosine-mediated vasodilation increased the size of the intravascular space, as reflected in the increase in tissue blood volume (Figure 4). Since adenosine did not increase O_2 consumption (Table 2), the decrease in tissue O_2 extraction and the increase in the tissue level of oxyhemoglobin and oxymyoglobin were expected (Figure 4).[34] The increase in the rate of oxyhemoglobin provision associated with adenosine-mediated hyperemia would have been expected to decrease the tissue level of deoxyhemoglobin. We found, however, that while adenosine increased blood flow and O_2 delivery by 82% and 107%, respectively, there was

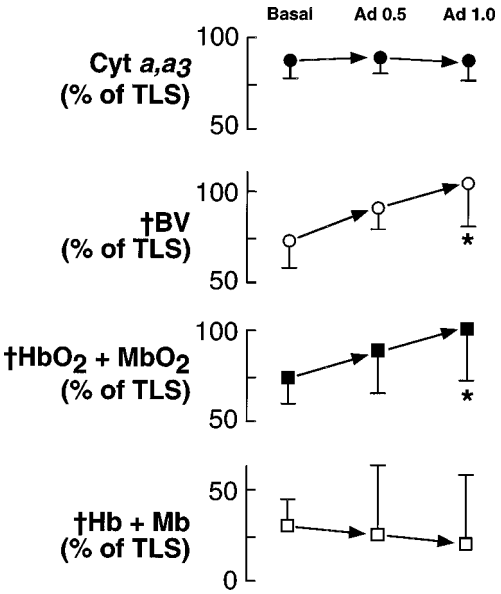

Fig. 4 NIR optical responses in canine myocardium prior to adenosine infusion (BASAL) and during infusion at 0.5 mg/kg/min (Ad 0.5) and 1.0 mg/kg/min (Ad 1.0). Signal levels are expressed as % of total labile signal (TLS). Data are presented as mean ± S.D. for seven dogs. Asterisk denotes $p<.05$ vs preinfusion levels. Abbreviations otherwise as in Figure 1.

not a significant decrease in the tHb+Mb level. Furthermore, the Cu_A oxidation level did not increase, implying that the relationship of electron flux to mitochondrial O_2 availability did not improve during adenosine infusion (Figure 4). Taken together, these data indicate that adenosine-mediated hyperemia does not augment transmural oxygen availability *in vivo*. Since adenosine-mediated hyperemia is known to be heterogeneous, the lack of improvement in myocardial oxygenation during adenosine infusion was not unexpected.[35] Though the precise mechanisms remain to be determined, one possibility is that pharmacologic doses of adenosine induce microvascular shunting. If so, adenosine infusion could alter the distribution of oxygen to metabolically heterogeneous regions, producing little or no augmentation in transmural oxygenation despite an increase in O_2 delivery.

The determinants of the magnitude and duration of reactive hyperemia in response to transient myocardial ischemia are multifactorial.[23,6] This vascular effect is due in part to (1) filling of the vascular tree and coronary driving pressure[36,21] myogenic relaxation[37,38] adenosine[30,23,31,19] other unrecognized factors causing vasodilation in response to myocardial metabolic demand (related to the duration of ischemia).[15,39–41,23] The adenosine hypothesis of metabolic coronary flow regulation proposes that blood flow is adjusted to myocardial oxygen requirements by the augmented formation of adenosine.[38] The data presented here indicate that coronary blood flow and O_2 extraction are indeed adjusted to myocardial oxygen requirements. The

results of this study, however, do not support the view that adenosine, at pharmacologic doses, has a beneficial effect on myocardial oxygenation. Furthermore, our results and the results of other studies[42,3,6] indicate that metabolic recovery occurs early during reactive hyperemia, prior to the reported onset of adenosine's vasodilatory effect *in vivo*.[30,23,31]

In summary, NIR spectroscopy demonstrates that cardiac O_2 extraction is increased and transmural O_2 availability is below preocclusion levels early during reactive hyperemia, whereas during the remainder of hyperemia, O_2 extraction is subnormal and O_2 availability is supranormal. Increasing the duration of coronary occlusion produces changes in myocardial O_2 availability and tissue blood volume during reperfusion which indicate that myocardial oxygenation is highly regulated after brief ischemia. Since cellular oxygen supply and recovery of oxidative metabolism probably are restored early during reactive hyperemia, the predominant effect of cellular metabolic demand on postischemic vasodilation should occur early in reperfusion. In contrast to during ischemia, MVO_2 during reactive hyperemia is not limited by O_2 delivery or cellular O_2 availability. The factors mediating the early vasodilator response to metabolic demand after brief ischemia, as well as the factors controlling the rate of myocardial respiration during reperfusion remain to be determined.

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