Noninvasive Orthogonal Polarization Spectral Imaging as Applied to Microvascular Studies in Mice

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In vivo observations of the mouse microcirculation can hardly be performed due to technical difficulties, limiting the knowledge that could be obtained from gene manipulated mice models. The aim of the present study was to check the applicability of a novel optical system, the orthogonal polarization spectral technology, to study the mouse microcirculation. In anaesthetized mice, the spinotrapezius muscle microcirculation was observed in situ. The diameter of precapillary arterioles was measured before and after a pharmacological or hormonal stimulation. High-contrast images of the muscle microcirculation were obtained and significant vasodilatation of arterioles was observed after topical applications of acetylcholine, sodium nitroprusside, and insulin. As compared to conventional techniques, orthogonal polarization spectral imaging makes it possible to assess and study microvascular beds in mice, which were inaccessible until now, allowing the use of gene manipulated mice to investigate, for example, the mechanisms involved in the development of diabetic microangiopathy.

Keywords
Arteriole; Microcirculation; Mouse; Orthogonal Polarization Spectral Imaging; Skeletal Muscle

Microvascular pathologies are a key feature of many human disease states, including hypertension [1], coronary heart disease [2], and diabetes mellitus [3]. In diabetes, the so-called diabetic microangiopathy underlies retinopathy, nephropathy, and neuropathy. It is well recognized that these long-term complications of diabetes originate from malfunction of the microvasculature [4]. In industrialized countries, diabetic retinopathy remains the leading cause of blindness [5], diabetic nephropathy is the main cause of end-stage renal disease [6], and diabetic peripheral neuropathies are the major cause of lower limb amputation [7]. These data highlight the urgent need to clarify the processes involved in the pathogenesis of diabetic microvascular complications in order to achieve efficient therapeutic strategies that can improve the microcirculatory function. However, the etiology of long-term diabetic microvascular complications is still poorly understood and this lack of knowledge can partly be explained by the technical difficulties linked to the observation of the microvasculature in situ [8].

Microcirculation is difficult to access because of its small dimensions and its location and therefore requires adapted techniques. In humans, conventional techniques available to observe the microcirculation are necessarily noninvasive, therefore limiting the investigations to vascular beds where vessels are visible and close to the surface such as skin, finger nail fold, or conjunctiva. However, this raises the question whether studies performed in these tissues are relevant for diabetes because (1) microvascular dysfunction during metabolic disorders occurs in many microvascular networks with more important physiological, functions, and (2) many tissues have their own physiological regulation. In animals, using invasive methods and video microscopy, deeper tissues can be observed, such as skeletal muscle (spinotrapezius, cremaster, extensor digitorum longus) or gastrointestinal tract (mesenter, colon). However, these microvascular preparations require extensive surgical procedures to exteriorize the organ, thus questioning their validity...
to represent the in vivo situation [9]. Classical microvascular preparations potentially exhibit also another drawback, because they are mostly impossible to adapt to animal species as small as mice. In vivo microcirculatory investigations are therefore currently limited to species such as rats or bigger animals. On the other hand, the field of metabolic disorders (insulin resistance, glucose intolerance, obesity, diabetes) has experienced an explosion of specific models over the past few years, which are essentially engineered by genetic manipulations in mice (protein overexpression or knockout). Investigating the microvascular modifications involved in the aggravation of metabolic dysfunction [8] or underlying diabetic vascular complications might increasingly require such models to unravel these functional connections.

Recently, a new technique for the imaging of microcirculation, the orthogonal polarization spectral (OPS) imaging [10], was developed. Compared to intraval microscopy, OPS imaging offered several advantages, making this device potentially advantageous to visualize the microcirculation in mice and to investigate quantitative parameters such as arteriolar diameter or functional capillary density changes [10]. Indeed, OPS imaging produces highly contrasted images of microvascular networks without the use of fluorescent dyes or elaborate and destructive tissue preparation for transillumination.

So, the aim of the present study was to check the applicability of the OPS imaging technology to study the mouse microcirculation when standard intraval microscopy (for example, transillumination) can hardly be performed. To this end, we adapted a previously described intact rat spinotrapezius muscle preparation [11] for the use in mice and validated its suitability for microvascular reactivity studies with vasoactive drugs and also its suitability for endocrine microvascular studies.

MATERIALS AND METHODS

Animals

Male C57Bl6/J mice (Charles River, l’Arbresle, France) weighing 26 ± 1 g were used for the experiments. The animals were kept under standard conditions (light 07.00 to 19.00 hours; temperature 22°C ± 1°C; humidity 50% ± 10%), and fed rodent diet and water ad libitum.

Spinotrapezius Muscle Preparation

All surgical and experimental procedures were performed under constant flow of isoflurane anaesthesia (2.0% to 2.5%) in oxygen–nitrous oxide gas mixture (40% to 60%). Throughout the whole experiment, body temperature was kept at 37°C ± 1°C by placing mouse in supine position on a complete homeothermic blanket system (Harvard Apparatus, Les Ulis, France). Polyethylene catheters (PE-10; Intramedic, FLD, Chilly Mazarin, France) were inserted into the carotid artery for blood pressure measurement. The rat spinotrapezius muscle preparation [11] was adapted and simplified for use in mouse: a longitudinal slit was performed in the skin along the spine from the cervical to the midlumbar region and the connective tissue that covers the muscle was carefully removed using iridectomy scissors. During the preparation and experimental observations, the skeletal tissue was continuously superfused with a bicarbonate/HEPES-buffered saline. The temperature of the solution was kept at 36°C and the pH was set at 7.4 by bubbling the solution with 5% CO2 in 95% N2. Superfusion flow rate was maintained at 4 to 5 mL/min (Figure 1).

Orthogonal Polarization Spectral Imaging Technology

The OPS imaging technology, described in detail by Groner and colleagues [10], allowed visualizing the microcirculation. Briefly, the technique eliminates directly reflected green polarized light from an organ surface using an orthogonal placed analyzer, which results in clear intraval images of red blood cells flowing through the microcirculation. For our study, we used the OPS imaging Cytoscan Video Microscope (Cytometric, Philadelphia, USA) with the 10× probe positioned on the top of the muscle that allowed the observation of microcirculatory fields of 0.47 × 0.63 mm. Images were captured and recorded on S-VHS videotape and analyzed off-line.

Image Analysis

Arteriolar diameters were determined by measurements of the red blood column diameters, the median value of at least 5 consecutive diameter measurements being considered as the real diameter. This was performed by a playback analysis of the video record using an image processing system. The image was coded as 768 by 572 pixels, with 256 gray levels. The isotropic orientation of the vessel was used to increase the signal/noise ratio, by integration of the segments perpendicular to the operator’s defined line drawn on the vessel. The diameters were computed at 50% of the gray level amplitude of the integrated signal.

Blood Pressure Measurements

The arterial blood pressure signal, transmitted to a blood pressure analyser (AH 60-3003, Harvard Apparatus, Les Ulis, France), was digitized at 1000 samples per second. Each second, mean blood pressure and heart rate were computed, displayed, and stored using dedicated software IOX (EMKA Technology, Paris, France).
Experimental Protocols

Mice were allowed a 45-minute stabilization period before the experimental protocols were begun. Four regions of interest within the spinotrapezius muscle that contained one or more terminal precapillary arterioles (smaller than 20 µm) were selected. For measurement, the 4 microcirculatory fields were recorded for ~60 seconds each; the value of arteriolar diameter at this time will be considered as basal diameter.

In a first series of experiments, we examined responses of mouse spinotrapezius arterioles to the endothelium-dependent vasodilator acetylcholine (Ach; 0.1 µ and 1.0 µ; n = 4). We also examined dilation of arterioles to the endothelium-independent nitric oxide donor sodium nitroprusside (SNP; 0.1 µM and 1.0 µM; n = 4). After baseline measurements, superfusion with bicarbonate buffer was interrupted and acetylcholine or sodium nitroprusside prepared in the same medium was topically applied. Arteriolar diameters were measured after 3 minutes of the topical application. In pilot experiments, maximal vasodilatory effects were obtained at the concentration of 1 µM. Thus, the concentration of 0.1 µM and 1.0 µM were selected in each case.

In a second series of experiments, mice were randomized into either the control group (n = 8) or insulin group (n = 8). In mice of the control group, physiological buffer solution was superfused throughout the entire experiment. In mice of the insulin group, 200 µIU/mL insulin (Actrapid, Novo Nordisk Pharmaceutique S.A., Boulogne, France) diluted in physiological buffer, was superfused for 45 minutes. Video recording was done at 15, 30, and 45 minutes after the start of superfusion for later off-line analysis.

Statistical Analysis

Results are expressed as mean ± SEM. Between-group comparisons of mice metabolic parameters were performed using
the Student-Newman-Keuls’ post hoc pairwise test. The data for arteriolar diameters were analyzed using 2-way analysis of variance (ANOVA) to identify time and drug effects. When a significant value was demonstrated, 1-way ANOVA was used, followed by the Tukey’s post hoc analysis, to analyze the time effect in each experimental group and drug effect for each series of experiment. A $P$ value of .05 or less was considered statistically significant. Data were analyzed using the statistical software SPSS (SPSS France, Paris, France).

RESULTS

OPS Imaging

High-contrast images of terminal precapillary arterioles of the mouse spinotrapezius muscle were obtained by the OPS imaging system (Figure 2), allowing the measurement of vascular diameter.

Baseline Data of the Mouse Spinotrapezius Model

Basal physiologic parameters of C57Bl6/J mice were similar in all groups: mean arterial blood pressure was 90 ± 5 mm Hg, heart rate was 545 ± 13 bpm, and precapillary arteriolar diameter established at 10.4 ± 0.5 µm. No significant changes of mean blood pressure and heart rate were observed during the experiments.

Effect of Vasoactive Drugs on Arteriolar Diameters

Figure 3 summarizes the effect of topical application of acetylcholine and sodium nitroprusside on the precapillary arteriolar diameter. Acetylcholine and sodium nitroprusside produced dose-related dilation of arterioles. Topical acetylcholine (0.1 µM and 1.0 µM) caused ∼20% and ∼35% increases over the initial arteriolar diameters, respectively, whereas topical sodium nitroprusside (0.1 µM and 1.0 µM) caused ∼25% and ∼45% increases over the initial arteriolar diameters, respectively.

Effect of Insulin on Precapillary Arteriolar Diameters

Arteriolar diameters in the spinotrapezius muscle remained stable over time in animals injected with isotonic saline. On the contrary, a pronounced vasodilation was induced by insulin (Figure 4). Precapillary arteriolar diameters were significantly increased at time 15 minutes (8.5%) and the effect persisted until the end of the experiment to reach the value of 12.3% at time 45 minutes.

DISCUSSION

The basic rationale for this study emerged from the facts that the pathophysiology of diabetic microangiopathy is still poorly understood. To fully understand the functional modifications

FIGURE 2
Representative examples of high-contrast images obtained in vivo by OPS imaging (Cytoscan Video Microscope) of the mouse spinotrapezius muscle arterioles (a) and venules (b) before (A) and after (B) topical application of acetylcholine (10 µM). Scale bar represent 100 µm.
FIGURE 3
Response of the mouse spinotrapezius muscles precapillary arterioles to topical application of acetylcholine (gray bars) and sodium nitroprusside (black bars). Data are means ± SEM.

FIGURE 4
Effects of insulin on the skeletal muscle precapillary arteriolar diameters in mice. Insulin (gray bars) was superfused at a dose of 200 µIU/mL over 45 minutes. In control mice (white bars), physiological buffer was superfused throughout the whole experiment. Data are means ± SEM. *P < .05 versus saline, †P < 0.05 versus time 0.
In summary, we examined the transfer and applicability of the OPS imaging technology to investigate the mouse microcirculation. As compared to conventional technique/models, OPS makes it possible to greatly reduce the level of surgery to access the microvascular bed of skeletal muscles, a tissue of primary importance in the pathophysiology of metabolic disorders. This technology will now provide a unique opportunity by allowing investigation of the pathophysiology of diabetic microangiopathy as an extension to gene manipulated mice.

REFERENCES


