Acute hyperglycemia depresses arteriolar NO formation in skeletal muscle

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Lash, J. M., G. P. Nase, and H. G. Bohlen. Acute hyperglycemia depresses arteriolar NO formation in skeletal muscle. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1513–H1520, 1999.—In the rat intestinal and cerebral microvascularities, acute D-glucose hyperglycemia suppresses endothelium-dependent dilation to ACh without affecting endothelium-independent dilation to nitroprusside. This study determined whether acute hyperglycemia suppressed arteriolar wall nitric oxide concentration ([NO]) at rest or during ACh stimulation and inhibited nitroprusside-, ACh- or contraction-induced dilation of rat spinotrapezius arterioles. Vascular responses were measured before and after 1-h topical 300 mg/100 ml D-glucose; arteriolar [NO] was measured with NO-sensitive microelectrodes. Arteriolar dilation to ACh was not significantly altered after superfusion of 300 mg/100 ml D-glucose. However, after hyperglycemia, arteriolar [NO] was not increased by ACh, compared with a 300 nM increase attained during normoglycemia. Arteriolar dilation to submaximal nitroprusside and muscle contractions was enhanced by hyperglycemia. These results indicated that in the rat spinotrapezius muscle, acute hyperglycemia suppressed arteriolar NO production while simultaneously augmenting vascular smooth muscle responsiveness to nitroprusside, presumably through cGMP-mediated mechanisms. In effect, this may have allowed ACh- and muscle contraction-induced vasodilation to be maintained during hyperglycemia despite an impaired NO system.

sodium nitroprusside; acetylcholine; arterioles

ABNORMAL ENDOTHELIAL CELL morphology and function have been reported early in the progression of insulin-dependent and -independent diabetes in humans (1, 8), rats (13, 16, 17), and rabbits (24). In streptozotocin-induced diabetic rats, brain endothelial cells became morphologically abnormal within 3 wk of chronic hyperglycemia, and ~20% of the cells appeared to be dying (17). Similar changes in endothelial cell morphology have been described in severely diabetic humans (1). In vitro studies using insulin-dependent diabetic rat arterioles (16) and rabbit aorta (24) have demonstrated that 6–10 wk of chronic hyperglycemia caused a significant suppression of endothelium-mediated vasodilation. Consistent with the above findings, in vivo studies in the intestinal microvasculature of insulin-dependent diabetic rats have shown that endothelium-dependent arteriolar dilation to acetylcholine is severely depressed within 1 wk of chronic hyperglycemia (13). In the above studies (13, 24), vasodilation to sodium nitroprusside, a cGMP-dependent vasodilator, was essentially normal. This finding would suggest an endothelial rather than a smooth muscle deficit during chronic hyperglycemia.

More recent studies suggest that acute exposure to hyperglycemia can have deleterious effects on endothelial cell structure and vasodilator function in diabetic and normal humans (26), rats (3, 9, 16), and rabbits (7). Salameh and colleagues (21) demonstrated in vitro that in normal human endothelial cells, exposure to hyperglycemia for ~36 h significantly increased giant and polynuclear cells and caused severe disruption of actin filaments. Pieper and Dondlinger (19) found that exposure of normal endothelial cells to hyperglycemia for ~24 h attenuated receptor-mediated production of endothelium-derived nitric oxide (NO). In vivo studies performed on both cerebral (16) and intestinal (4) arterioles have demonstrated that 1-h exposure to 300 mg/100 ml D-glucose decreased arteriolar responses to acetylcholine by >50% (4). In addition, in the study on intestinal arterioles (4), we found that pretreatment with superoxide dismutase, catalase, indomethacin, or meclofenamate preserved acetylcholine-induced vasodilation after acute hyperglycemia. These findings suggested that oxygen radicals derived from heightened prostaglandin formation during hyperglycemia attenuated endothelium-dependent arterial dilation. Interestingly, these same studies demonstrated that endothelium-independent dilation to sodium nitroprusside was not altered under these conditions, suggesting that vascular smooth muscle responsiveness to cGMP stimuli was normal during acute bouts of hyperglycemia.

To our knowledge, there have been no investigations into the effect of acute hyperglycemia on arteriolar NO concentration in vivo in the intact microvascular network. All of the current knowledge concerning the deleterious actions of hyperglycemia on endothelial cell function has been elucidated from the response of arteries and arterioles to endothelium-dependent agonists before and after hyperglycemia. In addition, it is unknown whether endothelium-mediated regulation of the skeletal muscle microvasculature is as disrupted by acute hyperglycemia as are the cerebral (15, 16) and intestinal (4) vasculatures. Therefore, the aim of the current study was to determine the effect that acute hyperglycemia has on basal arteriolar NO concentration ([NO]) and endothelium-dependent dilation to acetylcholine. A second aim of this study was to determine whether acute hyperglycemia alters endothelium-independent or functional vasodilation of these intact microvessels. To our surprise, acute exposure to 300 mg/100 ml D-glucose significantly enhanced functional...
dilation to muscle contraction and did not adversely affect endothelium-dependent dilation to acetylcholine or endothelium-independent dilation to nitroprusside or diazoxide. However, by combining direct measurement of arteriolar [NO] with NO-sensitive micro-electrodes and pharmacological blockade of NO and vasodilator prostanoid production, we were able to demonstrate that NO formation is depressed by hyperglycemia, and cGMP-mediated dilation is augmented. In effect, the hyperglycemia-induced endothelial cell impairment in the skeletal microcirculation mirrored that of cerebral and intestinal arterioles. However, hyperglycemia-induced alterations in vascular smooth muscle cell responsiveness to cGMP stimuli was quite different from that seen in other tissues.

METHODS

Surgical preparation of spinotrapezius. Adult male Sprague-Dawley rats (27 rats, 215–300 g; Harlan, Indianapolis, IN) were used in these experiments. Each animal was anesthetized with thiopental sodium (200 mg/kg; Abbott, Chicago, IL) injected subcutaneously at four locations over the thighs and lower back and placed on a heating mat to maintain a 37°C rectal temperature. One-fourth of the original dose was given intraperitoneally if supplemental anesthesia was needed. The trachea was intubated (PE-240) to ensure a patent airway, and the left femoral artery was cannulated (PE-50) for the subsequent monitoring of blood pressure. Experiments were conducted only if mean arterial pressure was >90 mmHg and stable.

The right spinotrapezius muscle was exteriorized as previously described (12). With this approach, the muscle was gently drawn away from the body wall without disturbing any of the feed vessels or neural inputs. Once freed of connective tissue, the muscle was secured with silk ligatures over a transparent pedestal. The in situ muscle length and width were measured before surgical exteriorization and reproduced by arrangement of the anchoring sutures. A superfusion chamber was then placed over the muscle, and the muscle was continuously superfused with a bicarbonate-based physiological solution (in mM: 119 NaCl, 6 KCl, 3.3 CaCl2, and 25 NaHCO3) equilibrated with 5% CO2–95% N2 and warmed to ~35°C, the in situ temperature of this superficial muscle.

After the spinotrapezius was exteriorized, the rat was transferred to the stage of an Olympus BHM intravalve microscope (Hyde Park, NY) that was connected to a charge-coupled device video camera (CCD-200-R video camera, Videoscope International, Washington, DC). Arcade arterioles (48–69 µm ID) from the spinotrapezius microcirculation were chosen for study. Video images of the arterioles were displayed on a Sony high-resolution video monitor (model PVM-1342Q Tokyo, Japan), digitized, and stored for analysis using the Image-1 acquisition and analysis software (Universal Imaging, West Chester, PA). The system was calibrated using the image of a stage micrometer marked with 10- and 100-µm increments. Dimension calibrations were performed in both the X and Y directions to account for any spatial distortion inherent to the system. Measurements of arteriolar luminal diameters were made from the digitized images using the superimposed caliper measuring function of the Image-1 software.

Experimental protocols. The first series of experiments was designed to determine the effect of acute hyperglycemia on endothelium-dependent, endothelium-independent, and functional dilation of spinotrapezius arterioles. To accomplish this, responses of arcade arterioles to locally applied acetylcholine, sodium nitroprusside, diazoxide, and skeletal muscle contraction were measured before and then after 1 h of topical hyperglycemia (isotonic D-glucose solution of 100, 200, or 300 mg/100 ml).

For iontophoresis, glass micropipettes (10- to 20-µm outer tip diameter) were filled with the appropriate drug dissolved in distilled water and connected to an iontophoresis current programmer (model 260, World Precision Instruments, Sarasota, FL). A retaining current of 20–80 nA was used to prevent release of the drug from the pipette during control conditions. The pipette was placed as close to the outer edge of the vessel wall as possible without dimpling the vessel wall surface or causing a local vascular response. During application of the respective drug, the position of the micropipette tip was adjusted as needed to maintain the original micropipette-vessel wall relationship. Incremental increases in the current dose were applied in sequence, and diameter responses were measured after 90–120 s to allow for development of the steady-state response. Pipette drug concentrations were 5 × 10–6 M for acetylcholine and 2 × 10–4 M for sodium nitroprusside. Application currents for both drugs were 10, 25, 50, 100, and 200 nA. These dosage parameters were determined in preliminary experiments to elicit threshold to maximum dilatory responses for the vessels studied.

Diazoxide, a selective ATP-dependent K+ channel agonist (20), was locally applied via microsuffusion. For microsuffusion, glass micropipettes (10- to 14-µm outer tip diameter) were filled with 10–3 M diazoxide in our physiological saline and connected to a precision micropump system (model 1400, World Precision Instruments). The micropipette tip was placed in light contact with the vessel, and progressive elevations in diazoxide dosage were applied in sequence by step increases in pump flow rate (2.5, 5, and 10 nl/min). The arteriolar diameter responses at each diazoxide dosage were measured during steady-state responses.

For muscle contractions, needle-style, nontraumatic stimulating electrodes were placed at the rostral and caudal ends of the muscle and attached to a Grass SD-9 stimulator. Stimulus parameters of 0.2-ms duration and ~8 V were selected such that muscle contractions were elicited through stimulation of the motor neurons, and stimulus pulses were maintained below the threshold for direct vascular effects (12). Diameter responses to muscle contractions were evaluated at the same vessel location observed during iontophoretic application of acetylcholine and nitroprusside. Images were obtained in the resting muscle and at 2-min intervals as contraction frequency was increased to 2, 4, and 8 Hz. Previous studies have demonstrated that steady-state functional vasodilation is achieved within ~90 s after the onset of muscle contraction and is maintained for >5 min of total contraction time (12). Furthermore, 8-Hz contractions have been shown to elicit near-maximal dilation of rat spinotrapezius muscle arterioles (12). A 10-min recovery time was allowed following muscle contractions before subsequent experimental measurements were obtained. Under control conditions, initial resting diameters were consistently retested within the allotted recovery time.

After data for normal conditions were collected, isotonic D-glucose solution was added to the standard physiological solution to establish a final glucose concentration of 100, 200, or 300 mg/100 ml. After 1 h of D-glucose superfusion, responses of arcade arterioles to locally applied acetylcholine, sodium nitroprusside, diazoxide, and muscle contraction were retested. D-glucose remained in the bathing fluid throughout the experiment.
A second series of experiments was designed to determine whether endogenous NO and/or vasodilator prostaglandins are modulators of endothelium-dependent or -independent vasodilation during acute hyperglycemia. Previous experiments in the rat small intestine have indicated that the microvascular effects of acute hyperglycemia were minimized during blockade of cyclooxygenase with indomethacin or meclofenamate (4). To determine whether a similar protective function occurs in skeletal muscle, responses of arcade arterioles to iontophoresically applied acetylcholine or nitroprusside were measured during resting conditions, after 1 h of topical hyperglycemia (D-glucose 300 mg/100 ml), and after subsequent addition of the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 10−4 M) or the cyclooxygenase inhibitor meclofenamate (3 × 10−5 M).

For iontophoresis, application of acetylcholine or nitroprusside was identical to the protocol described for the first series of experiments. After iontophoretic application of the respective drug, isotonic 300 mg/100 ml D-glucose was added to the standard physiological solution. After 1 h of D-glucose superfusion, responses of arcade arterioles to iontophoresically applied acetylcholine or nitroprusside were retested. After the last dosage of the respective drug, L-NAME was added to the physiological saline at a final concentration of 10−4 M (14). After an equilibration period of ~20 min, arteriolar responses to acetylcholine or nitroprusside were evaluated. After a 30-min washout period, meclofenamate was added to the physiological saline for 30 min at a final concentration of 3 × 10−5 M (4), and arteriolar responses to the respective drug were retested one last time. D-glucose remained in the bathing fluid throughout the experiment.

In a third series of experiments, we evaluated the effect of acute hyperglycemia on arteriolar NO concentration ([NO]) and arteriolar diameter during resting conditions and after microsuffusion of acetylcholine. To accomplish this, arteriolar [NO] was measured with a NO-sensitive microelectrode, and arteriolar diameter was measured during resting conditions and during microsuffusion of acetylcholine before and 1 h after acute hyperglycemia (300 mg/100 ml).

For measurement of arteriolar [NO], NO-sensitive, recessed-tip microelectrodes were used based on the techniques developed by Bleck and colleagues (5). The NO microelectrodes had a 7- to 13-µm outer tip diameter after being sharpened and a 50- to 70-µm open tip recess; 50–100 µm of gold were plated distal to the recess. The electrode recess was coated with 5% Nafion (Aldrich Chemical, Milwaukee, WI) to exclude anions that might induce an artifactual signal. A World Precision Instruments tonometer held at 34.5 ± 0.5°C was used to calibrate the microelectrodes at three known [NO]. A custom-designed electrometer based on an Analog Devices AD515 microchip was used to measure picocammere currents. The electrodes were polarized at 0.8 V, with the electrode positive relative to a carbon fiber electrode (World Precision Instruments). Nitrogen-equilibrated saline was used as a zero reference for NO, and ~362 and 798 parts per million NO in nitrogen were used to establish a 0–1,200 nM calibration range. The microelectrode currents were measured when the current became stable. All electrodes used for data collection were found to have a linear relationship of electrical current to [NO]. Electrodes typically had a current of 12–15 pA in nitrogen-equilibrated saline and for 1,000 nM [NO], generated an additional 1.0–2.0 pA.

Previous in vivo and in vitro testing has demonstrated that these NO microelectrodes are very sensitive and selective to NO (3, 5). Briefly, in these past studies, NO-sensitive microelectrodes did not respond significantly to physiological concentrations of norepinephrine and ascorbic acid, and in the current study, the microelectrodes did not respond to 200 µM tyrosine; all these biological agents oxidize at about the same polarization voltage range (~0.05–1 V) as NO. Furthermore, NO microelectrodes respond to changes in [NO] during in vivo conditions that were consistent with the findings for NO production in bioassay experiments (3). Topical exposure of the intestinal arterioles to 1 mM L-arginine doubled the [NO] measured by the NO microelectrode, and subsequent addition of 0.5 mM Nω-nitro-L-arginine decreased [NO] to about one-half of the resting [NO] (3). The NO-sensitive microelectrodes were very stable over a period of three to four experiments. After each experiment, NO microelectrode sensitivity to NO was retested in the calibration system and generally found to change less than ±10%.

The electrodes were extremely sensitive to any fluctuation in bath temperature (>1°C). To avoid consequences of the thermal sensitivity of the electrode, the bath temperature above the tissue was the same as the tissue for a depth of 2–3 mm. The electrodes were held at a 15° angle to the tissue so that 5 mm of the shaft and the entire tapered tip was thermally protected. The electrode tip was moved ~1 mm above the tissue surface to establish a 0 nM reference for [NO]. The arteriolar wall [NO] was measured by lightly pressing the NO microelectrode tip against the outer vessel wall. After the electrode tip was properly positioned, the vessel and NO microelectrode were allowed to stabilize for ~5 min. If the electrode tip perturbed the vessel such that dilation or constriction occurred, the local [NO] usually increased for ~5–10 min before baseline values were regained. The perturbance offset should be avoided for reliable measurement of [NO].

After [NO] was measured during resting conditions, acetylcholine was microsuffused locally over the arcade arteriole, and [NO] was remeasured along with arteriolar luminal diameter. Microsuffusion of acetylcholine was used during the protocol to measure [NO] because iontophoretic application of acetylcholine caused only a very localized increase in [NO] near the tip of the iontophoresis pipette. In contrast, microsuffusion of acetylcholine caused a uniform increase in [NO] over a larger length of the arteriole (~100 µm). For microsuffusion, glass micropipettes (6– to 8-µm outer tip diameter) were filled with 5 × 10−3 M acetylcholine in physiological saline and were connected to a precision micro-pump system. The NO microelectrode sensor tip was not sensitive to either acetylcholine or microsuffusion of the vehicle at the highest flow rate possible (100 nl/min). The micropipette tip was placed in light contact with the tissue, and a negative retaining flow of 0.5 ml/min was used to prevent release of the drug by diffusion or hydrostatic gravity forces. Perfusion of the bathing media from the micropipette tip did not cause vascular responses. Progressive elevations in acetylcholine doses were applied in sequence by step increases in pump flow rate (5, 10, 15, 20, and 25 nl/min). The arteriolar [NO] and luminal diameter responses at each acetylcholine dosage were measured during the steady-state responses. After microsuffusion of acetylcholine, isotonic D-glucose solution was added to the standard physiological solution to achieve a glucose concentration of 300 mg/100 ml. The NO microelectrodes were insensitive to bath addition of 100–300 mg/100 ml D-glucose. After 1 h of D-glucose superfusion, arteriolar [NO] and arteriolar diameter responses of arcade arterioles to microsuffused acetylcholine were retested. D-glucose remained in the bathing fluid throughout the experiment.

Statistical analysis. All data are expressed as means ± SE, and statistical analysis was carried out using Sigma Stat (Jandel Scientific, San Rafael, CA). Repeated measures proce-
Arteriolar nitric oxide and hyperglycemia

Effect of acute hyperglycemia on acetylcholine-, nitroprusside-, diazoxide-, and muscle contraction-induced vasodilation. For this section of the study, 11 male Sprague-Dawley rats (231 ± 9 g) were used. The mean resting diameter of arcade arterioles was 60 ± 5 µm, and the diameters following D-glucose solutions of 100, 200, or 300 mg/100 ml were not significantly changed (62 ± 7, 59 ± 4, and 57 ± 8 µm, respectively).

We found that superfusion with 100 mg/100 ml D-glucose (data not shown) or 200 mg/100 ml D-glucose (Figs. 1 and 2, top) did not alter vascular responsiveness to either acetylcholine or nitroprusside. However, 200 mg/100 ml D-glucose did enhance functional vasodilation in response to low-frequency muscle contraction (2 Hz) by −130% (Fig. 1, bottom). After 1 h of superfusion with 300 mg/100 ml D-glucose, vascular responses to acetylcholine, nitroprusside, and muscle contraction were augmented. These data are presented in the bottom panels of Figs. 1, 2, and 3, respectively. As shown in Fig. 1 (bottom), iontophoretic application of acetylcholine at 10, 25, 50, 100, and 200 nA induced arteriolar dilations of 10 ± 6, 32 ± 7, 50 ± 7, 65 ± 9, and 77 ± 8 µm under the normal superfusate versus 43 ± 5, 66 ± 8, 82 ± 9, 86 ± 8, and 86 ± 8 µm in the presence of 300 mg/100 ml D-glucose. The data in Fig. 2 (bottom) demonstrate that iontophoretic application of nitroprusside at 10, 25, 50, 100, and 200 nA induced arteriolar dilations of 1 ± 1, 30 ± 5, 48 ± 8, 72 ± 9, and 78 ± 9 µm under the normal superfusate versus 16 ± 5, 44 ± 7, 72 ± 6, 75 ± 6, and 76 ± 6 µm in the presence of 300 mg/100 ml D-glucose. As shown in Fig. 3, muscle contractions at 2, 4, and 8 Hz induced functional arteriolar dilation of 5 ± 2, 72 ± 7, and 89 ± 6 µm under the normal superfusate versus 79 ± 8, 90 ± 7, and 90 ± 5 µm in the presence of 300 mg/100 ml D-glucose. As demonstrated in Fig. 4, vasodilatory responses to diazoxide were unaffected during acute hyperglycemia.

Effect of L-NAME and medofenamate on endothelium-dependent and -independent vasodilation during hyperglycemia. For the following experiments, 14 male Sprague-Dawley rats (231 ± 12 g) were used. The mean arteriolar resting diameter was 55 ± 6 µm, and the diameters following superfusion with 300 mg/100 ml D-glucose, L-NAME, or medofenamate were not significantly altered (56 ± 7, 57 ± 5, and 58 ± 7 µm,
respectively). As shown in Fig. 5, acetylcholine-induced vasodilation at 25, 50, and 100 nA was significantly increased during acute hyperglycemia. However, subsequent blockade of NO synthase, cyclooxygenase, or both had no effect on the hyperglycemia-induced augmented vasodilation to acetylcholine. Data in Fig. 6 demonstrate that nitroprusside-induced vasodilation at 25, 50, and 100 nA was also significantly increased during acute hyperglycemia. Furthermore, blockade of NO synthase or cyclooxygenase had no effect on the hyperglycemia-induced augmented vasodilation to nitroprusside.

**DISCUSSION**

The principal finding of this study is that acute topical 300 mg/100 ml hyperglycemia significantly de-
pressed basal arteriolar \([NO]\) and acetylcholine-induced \([NO]\) in the rat spinotrapezius muscle. The deleterious action of \(\alpha\)-glucose hyperglycemia on NO production during acetylcholine stimulation supports our previous findings for intestinal arterioles (4) and those of Mayhan and colleagues (15) for cerebral arterioles. However, despite the reduced ability of skeletal muscle arterioles to produce NO after hyperglycemia, these microvessels maintained their dilatory response to acetylcholine. Furthermore, vasodilation to nitroprusside (Fig. 2) and muscle contractions (Fig. 3) were both significantly augmented during hyperglycemia. The finding that acetylcholine-mediated vasodilation was not impaired (Figs. 1 and 7) and that nitroprusside-mediated vasodilation was augmented (Fig. 2) is unique and contrary to the results obtained in other vascular beds. During acute hyperglycemia, vascular relaxation to acetylcholine and other endothelium-dependent vasodilators was reported to be depressed in the human forearm (26), rabbit aorta (7), rat intestine (4), mesentery (23), and brain (15), whereas cGMP-mediated vasodilation was found to be normal. In experiments performed before we were able to measure NO, we blocked NO formation with \(l\)-NAME after hyperglycemia and found that sustained dilatory responses to acetylcholine were not associated with NO formation (Fig. 5). However, even this approach did not fully convince us that NO formation was depressed under these conditions. Once we were able to measure the actual perivascular \([NO]\) with NO-sensitive microelectrodes and found that \([NO]\) did not increase during acetylcholine stimulation following hyperglycemia, we concluded that the dilation to acetylcholine under these conditions was mediated by a mechanism or vasoactive factor other than NO. Sustained dilation to acetylcholine during hyperglycemia is not mediated by vasodilator prostaglandins because meclofenamate did not alter acetylcholine-induced dilation following hyperglycemia (Fig. 5). We did find that arteriolar vasodilation to nitroprusside is increased after hyperglycemia (Fig. 6). When nitroprusside is exposed to tissue, we detect an increased \([NO]\) with the NO-sensitive microelectrodes and we assume that this NO is activating cGMP in the vascular smooth muscle. Therefore, the dilation caused by acetylcholine after hyperglycemia could act by a mechanism that uses the intact cGMP pathway.

Regarding the local application of acetylcholine, we did find that iontophoresis of acetylcholine to a very localized area of one arteriole caused greater vasodilation after hyperglycemia than did microsuffusion of acetylcholine over a longer length of an individual arteriole. In Figs. 1 and 5, acetylcholine was delivered via microiontophoresis, whereas in Fig. 7, acetylcholine was delivered locally through a micropipette that was connected to a micropump system. As stated in METH-
which corresponded to an average decrease of
induced dilation was not reversed by L-NAME (Fig. 5). Hyperglycemia was that the augmented acetylcholine-
in regression to NO synthase activity posthyperglycemia. Topical exposure of spinotrapezius arterioles to $10^{-4}$ M L-NAME, which has been shown to
cause maximal inhibition of acetylcholine-induced dilation in this vascular bed (14), had no effect on resting arteriolar diameter during acute hyperglycemia (Fig. 5). This finding is interesting because our laboratory (14), as well as other investigators (2, 18), have demonstrated that blockade of basal NO synthase activity in the normal rat spinotrapezius muscle caused arterioles to constrict $\sim 10$–20%. The absence of a constrictor effect of L-NAME on resting arteriolar diameter following 1 h of hyperglycemia suggested that basal NO production was depressed rather than enhanced during acute bouts of hyperglycemia. The further finding to suggest that NO production was depressed during hyperglycemia was that the augmented acetylcholine-induced dilation was not reversed by L-NAME (Fig. 5). Further evidence to confirm that NO production was impaired during hyperglycemia came from the direct measurement of arteriolar [NO] with NO-sensitive microelectrodes. The basal [NO] decreased by $\sim 17\%$ during acute exposure to 300 mg/100 ml D-glucose, which corresponded to an average decrease of $\sim 48$ nM from the paired normoglycemia concentration (Fig. 7). In addition, during hyperglycemia, microsuffusion of acetylcholine did not increase the arteriolar [NO] (Fig. 7). In contrast, a dose-dependent increase in arteriolar [NO] occurred in response to acetylcholine during normoglycemia (Fig. 7). To our knowledge, this is the first in vivo study to directly show that acute hyperglycemia suppressed basal arteriolar [NO] and attenuated acetylcholine-mediated increases in [NO] in the rat microvasculature. The NO-sensitive microelectrode cannot discriminate between a hyperglycemia-mediated decrease in NO production or an increase in NO destruction mediated by oxygen radicals known to be formed during acute hyperglycemia (4, 9). However, our finding that basal [NO] decreased and endothelium-dependent production of NO could not be increased during acute hyperglycemia is consistent with the deleterious actions of acute hyperglycemia on endothelial cell structure (1, 17, 21) and endothelial regulation of arteriolar tone (4, 7, 15, 23).

The above findings ruled out NO as a mediator of the sustained acetylcholine dilatory response during hyperglycemia. However, acetylcholine-induced vasodilation may be partly regulated by other vasoactive factors or enhanced vascular smooth muscle responsiveness to cGMP-mediated stimuli. In skeletal muscle, acetylcholine-mediated vasodilation can be attenuated by 45–65% through NO synthase inhibition (2, 14, 18), indicating that about half of this dilation was mediated by NO. The remaining dilatory response could represent either incomplete blockade of NO synthesis or the involvement of other vasoactive factors. In the current study, we were concerned that hyperglycemia might increase the contribution of prostaglandins to acetylcholine-induced vasodilation, even though Koller and Kaley (10) and Vicaut and colleagues (25) demonstrated that neither meclofenamate nor indomethacin influenced acetylcholine-induced arteriolar dilation in normal rat skeletal muscle. We found that meclofenamate, an inhibitor of prostaglandin formation, did not affect resting arteriolar diameter or acetylcholine-mediated vasodilation during hyperglycemia (Fig. 6). This finding argued against a role for vasodilator prostaglandins in mediating the augmented acetylcholine response seen during hyperglycemia.

Having ruled out NO and vasodilator prostaglandins as mediators of the sustained acetylcholine dilatory response during hyperglycemia, our finding of an augmented nitroprusside response during hyperglycemia strongly suggested that there was a change in vascular smooth muscle function under these conditions (Fig. 6). Furthermore, our finding that vascular smooth muscle responsiveness to diazoxide (Fig. 4), an ATP-dependent K$^+$ channel agonist, was not altered by acute hyperglycemia indicated that there was some degree of specificity to the hyperglycemia-induced alteration in vascular smooth muscle responsiveness. Sodium nitroprusside, which we have verified as an NO donor in vivo with NO-sensitive microelectrodes, is known to cause relaxation of vascular smooth muscle through endothelium-independent increases in cGMP formation (6, 22, 27). In effect, our findings suggested that following hyperglycemia, vascular smooth muscle cells in the spinotrapezius muscle relax to a greater extent in response to cGMP-mediated vasodilators. This may explain why functional vasodilation was increased at submaximal muscle contraction frequencies (Fig. 3). We have previously demonstrated that more than 60% of the functional dilation associated with contractions of the intact spinotrapezius muscle was dependent on an intact NO system (14). In the current study, we fully expected hyperglycemia to attenuate muscle contraction-induced functional dilation by impairing NO production. However, although the microvascular NO system is compromised under these conditions, functional vasodilation induced by muscle contractions was increased at contraction frequencies that normally cause submaximal dilation.
The finding of a normal to enhanced acetylcholine- and muscle contraction-induced vasodilation during hyperglycemia is unique to skeletal muscle and suggests that this vascular bed can compensate for an impaired NO system in diabetic patients who are routinely normoglycemic. Taken together, our findings indicate that although hyperglycemia suppressed the formation of NO during basal conditions, acetylcholine stimuli, compensated for increased vascular smooth muscle responsiveness to cGMP stimuli, and muscle contractions, other mechanisms, including simultaneous enhancement of vascular smooth muscle responsiveness to cGMP stimuli, compensated for the impaired NO system.

The authors gratefully acknowledge the expert technical assistance of Mary Ann Neil in this study.

This study was supported by National Heart, Lung, and Blood Institute Grants HL-20605 and HL-25824. G. Nase was supported by a postdoctoral fellowship from the Indiana affiliate of the American Heart Association.

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Received 18 September 1998; accepted in final form 24 May 1999.

REFERENCES