H₂O₂ increases production of constrictor prostaglandins in smooth muscle leading to enhanced arteriolar tone in Type 2 diabetic mice

Nóra Erdei,² Zsolt Bagi,² István Édes,² Gabor Kaley,¹ and Akos Koller¹,³
¹Department of Physiology, New York Medical College, Valhalla, New York; ²Division of Clinical Physiology, Institute of Cardiology, University of Debrecen, Debrecen, Hungary; and ³Department of Pathophysiology, Semmelweis University, Budapest, Hungary

Submitted 7 January 2006; accepted in final form 20 September 2006

H₂O₂ increases production of constrictor prostaglandins in smooth muscle leading to enhanced arteriolar tone in Type 2 diabetic mice. *Am J Physiol Heart Circ Physiol* 292: H649–H656, 2007. First published September 22, 2006; doi:10.1152/ajpheart.00596.2006.—Our previous study showed that arteriolar tone is enhanced in Type 2 diabetes mellitus (T2-DM) due to an increased level of constrictor prostaglandins. We hypothesized that, in mice with T2-DM, hydrogen peroxide (H₂O₂) is involved in the increased synthesis of constrictor prostaglandins, hence enhanced basal tone in skeletal muscle arterioles. Isolated, pressurized gracilis muscle arterioles (~100 µm in diameter) of mice with T2-DM (C57BL/KsJ-db/db) exhibited greater basal tone to increases in intraluminal pressure (20–120 mmHg) than that of control vessels (at 80 mmHg, control: 25 ± 5%; db/db: 34 ± 4%, P < 0.05), which was reduced back to control level by catalase (db/db: 24 ± 4%). Correspondingly, in carotid arteries of db/db mice, the level of dichlorofluorescein-detectable and catalase-sensitive H₂O₂ was significantly greater. In control arterioles, exogenous H₂O₂ (0.1–100 µmol/l) elicited dilations (maximum, 58 ± 10%), whereas in arterioles of db/db mice H₂O₂ caused constrictions (~28 ± 8%), which were converted to dilations (maximum, 16 ± 5%) by the thromboxane A₂/prostaglandin H₂ (TP) receptor antagonist SQ-29548. In addition, arteriolar constrictions in response to the TP receptor agonist U-46619 were not different between the two groups of vessels. Endothelium denudation did not significantly affect basal tone and H₂O₂-induced arteriolar responses in either control or db/db mice. Also, in arterioles of db/db mice, but not in controls, 3-nitrotyrosine staining was detected in the endothelial layer of vessels. Thus we propose that, in mice with T2-DM, arteriolar production of H₂O₂ is enhanced, which leads to increased synthesis of the constrictor prostaglandins thromboxane A₂/prostaglandin H₂ in the smooth muscle cells, which enhance basal arteriolar tone. These alterations may contribute to disturbed regulation of skeletal muscle blood flow in Type 2 diabetes mellitus.

arteriolar tone; thromboxane A₂; cyclooxygenase 2; 3-nitrotyrosine; db/db

**TYPE 2 DIABETES MELLITUS** is associated with a markedly increased incidence of cardiovascular diseases, accounting for ~70% of deaths in the diabetic population (40). However, the relationship between Type 2 diabetes and cardiovascular disease is not completely understood and has been the subject of some dispute. Previous studies have demonstrated that vaso-motor dysfunction of microvessels is an early manifestation of vascular complications (10, 19, 36), which may lead to disturbed regulation of tissue perfusion, predisposing diabetic patients to tissue ischemia, as well as early development of hypertension.

In Type 2 diabetes mellitus, an important role of reactive oxygen species (ROS) contributing to the impaired regulation of arteriolar tone has recently received a great deal of attention. It seems well established that an enhanced production of superoxide anion interferes with several endothelial mechanisms, such as nitric oxide (NO) mediated, leading to impaired endothelium-dependent vasodilation in subjects with Type 2 diabetes (8). In this context, in mice with Type 2 diabetes of genetic origin, previously, we have demonstrated a key role for an enhanced vascular production of superoxide anion, which reduced flow-induced dilations of coronary arterioles by interacting with endothelium-derived NO (5).

Much less is known regarding the interrelationship between enhanced ROS production and smooth muscle-dependent mechanisms regulating arteriolar tone in Type 2 diabetes mellitus. In a rat model of Type 2 diabetes, superoxide anion has been proposed to play a role in alterations of smooth muscle-dependent myogenic activation of skeletal muscle arterioles (12). Recently, we have demonstrated that skeletal muscle arterioles of Type 2 diabetic mice exhibit an enhanced basal tone due to the increased endogenous production of cyclooxygenase 2 (COX-2)-derived constrictor prostaglandins, namely, thromboxane A₂/prostaglandin H₂ (TXA₂/PGH₂) (2), although the relationship between enhanced ROS production and altered prostaglandin synthesis remains obscure.

Superoxide anion is a highly reactive molecule, but it is unstable, and its capacity for diffusion is limited. Superoxide is rapidly (within ~10⁻⁹ s) converted by superoxide dismutases to the still reactive, but much more stable, hydrogen peroxide (H₂O₂) (43). Although H₂O₂ is a non-radical form of ROS and only possesses moderate oxidant activity, it can easily diffuse across plasma membranes (35). Interestingly, recent studies (20, 26, 35, 37) propose an important role for H₂O₂ in the mediation of arteriolar responses both in physiological and in pathophysiological conditions. In these studies, H₂O₂ elicited constriction of rat mesenteric (13), skeletal muscle (9), and mouse tail arterioles (32), whereas it diluted human atrial (29) and cat and piglet pial arterioles (27, 41), implying that, if the concentration of H₂O₂ reaches a certain level, it can result in substantial vasmotor changes.

On the basis of previous studies, we hypothesized that, in the wall of skeletal muscle arterioles of Type 2 diabetic mice, the...
level of H_2O_2 is increased, which contributes to the TXA_2/PGH_2-mediated enhanced arteriolar tone (2).

METHODS

Animals and experimental procedures. In the experiments, a well-characterized mouse model of Type 2 diabetes mellitus was used (4, 5, 18). Twelve- to fourteen-week-old, male db/db (C57BL/KsJ-db/db) and heterozygous (C57BL/KsJ-db/db) mice were fed standard chow and had free access to water. All protocols were approved by the Institutional Animal Care and Use Committee at New York Medical College (Valhalla, NY). Mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). Under anesthesia, carotid artery and gracilis muscle were excised and placed in ice-cold physiological salt solution (PSS).

Isolation of gracilis muscle arteriole. Microsurgery instruments and an operating microscope were used for isolation of a gracilis muscle arteriole (~0.5 mm in length) running intramuscularly. The arteriole was isolated and transferred into an organ chamber containing two glass micropipettes filled with Krebs solution composed of (in mmol/l) 110 NaCl, 5.0 KCl, 2.5 CaCl_2, 1.0 MgSO_4, 1.0 KH_2PO_4, 5.0 glucose, and 24.0 NaHCO_3 equilibrated with a gas mixture of 10% O_2, 5% CO_2, balanced with nitrogen, at pH 7.4. Vessels were cannulated on both ends, and micropipettes were connected with silicone tubing to a pressure servo control system (Living Systems Instrumentation). Temperature was set at 37°C by a temperature controller. The internal arteriolar diameter at the midpoint of the arteriolar segment was measured by videomicroscopy with a microangiometer (Texas Instruments). Changes in arteriolar diameter and intraluminal pressure were continuously recorded with the Biopac-MP100 system connected to a computer and analyzed with AcqKnowledge data acquisition software (Biopac Systems) (4, 5).

Arteriolar tone as a function of pressure. After a 1-h incubation period, spontaneous basal arteriolar tone developed in response to 80-mmHg intraluminal pressure, without the use of any constrictor agent. Changes in the diameter of arterioles were then measured in response to step increases in intraluminal pressure from 20 to 120 mmHg. Arterioles were incubated with catalase (200 U/ml for 30 min) and pressure-induced diameter changes were reassessed. The pressure-diameter relationship was also observed after endothelium denudation. Endothelium was removed by perfusion of air, as described previously (22). Acetylcholine (10^{-7} mol/l) and the NO donor sodium nitroprusside (10^{-7} mol/l) were used to assess the efficacy of endothelium denudation. To obtain the passive arteriolar characteristics, pressure-induced arteriolar responses were measured in the presence of Ca^{2+}-free PSS. Normalized arteriolar diameter (in Ca^{2+}-containing PSS) was expressed as a percentage of corresponding passive diameters (in Ca^{2+}-free PSS).

Detection of H_2O_2 by fluorescence. Dichlororhydrinfluorescein (DCFH) was used to assess the vascular production of H_2O_2, as described previously (29). Four carotid arteries from control and four from db/db mice were cleared of connective tissue and placed on PSS. DCFH (5 × 10^{-6} mol/l) was added in a light-protected chamber for 30 min at 37°C either in the absence or presence of catalase (200 U/ml). Vessels were then washed with PSS and removed for fluorescence microscopy. DCFH was excited at 488 nm. Vessels were examined in parallel, and images were recorded by using the same gain and intensity settings. Images were measured for fluorescence intensity (with Image 1 analysis software) within the distal, middle, and proximal segments of arteries, and average intensities were compared in arteries of control (n = 4) and db/db mice (n = 4).

Arteriolar responses to exogenous H_2O_2. In separate experiments, in the presence of an intraluminal pressure of 80 mmHg, H_2O_2 (10^{-7}–10^{-4} mol/l) was added to the organ chamber, and diameter changes were continuously recorded. H_2O_2-induced responses were also obtained after endothelium-denudation or in the presence of the TXA_2/PGH_2 (TP) receptor antagonist SQ-29548 (10^{-6} mol/l). In separate protocols, arteriolar responses were measured in response to the TP receptor agonist U-46619 (10^{-9} mol/l to 3 × 10^{-7} mol/l), before and after SQ-29548 treatment.

Detection of peroxynitrite by immunohistochemistry. Gracilis muscle from control (n = 3) and db/db (n = 3) mice were embedded and frozen in OCT compound (Tissue Tek, Electron Microscopy Sciences). Acetone-fixed consecutive sections (10-μm thickness) were immunolabeled with a polyclonal antibody against 3-nitrotyrosine containing protein (dilution 1:100; Calbiochem). Immunostaining was performed by using avidin-biotin horseradish peroxidase visualization system (Vectastain kit, Vector Laboratories), stained with diaminobenzidine tetrahydrochloride. As a control for nonspecific binding, the primary antibody was omitted. Images of the sections were collected with a digital camera (model CFW-1310C, Scion) connected to a Nikon Eclipse 80 microscope.

Statistics. Data are expressed as means ± SE. Statistical analyses were performed by two-way analysis of variance for repeated measures followed by the Tukey post hoc test or Student’s t-test, as appropriate. P < 0.05 was considered statistically significant.

RESULTS

Previously, we have found that, at 12 wk of age, body weight, serum glucose, and serum insulin of db/db mice were significantly elevated when compared with age-matched control heterozygous animals (4, 5), resembling data obtained from patients with obesity and Type 2 diabetes.

Arteriolar tone as a function of intraluminal pressure. After a 1-h incubation period, spontaneous myogenic tone developed in isolated skeletal muscle arterioles without the use of any vasoactive agent. Stepwise increases in intraluminal pressure from 20 to 120 mmHg elicited significantly greater tone of arterioles from db/db mice when compared with control vessels at each pressure step (Fig. 1A). Incubation and presence of catalase did not affect the pressure-diameter curves of arterioles of control mice, whereas it shifted this curve significantly upward in arterioles of db/db mice, back to control levels (Fig. 1A). Endothelium denudation did not affect significantly the pressure-induced changes in diameter of arterioles in either group (Fig. 1B). The passive pressure-diameter curves of arterioles (obtained in Ca^{2+}-free solution) were not different in the two groups of animals (at 80 mmHg: control, 135 ± 9 μm vs. db/db, 140 ± 8 μm).

Detection of H_2O_2 by fluorescence. In carotid arteries of db/db mice, an enhanced DCFH fluorescence was detected, indicating an increased level of H_2O_2. Presence of catalase reduced DCFH fluorescence in arteries of db/db mice, whereas it did not affect fluorescence intensity in control vessels (Fig. 2, A and B).

Arteriolar responses to exogenous H_2O_2. In separate experiments, arteriolar responses were obtained to exogenously administered H_2O_2 in the two groups of animals. H_2O_2, in a dose-dependent manner (10^{-7} to 10^{-4} mol/l), elicited substantial dilations in control arterioles, but it caused constrictions in db/db arterioles (Fig. 3, A and B). Endothelium-denudation did not affect significantly H_2O_2-induced differences in arteriolar responses in the two groups of animals (Fig. 3C). Presence of the TP receptor antagonist SQ-29548 (10^{-6} mol/l) did not significantly affect H_2O_2-mediated dilations of control arterioles. However, SQ-29548 converted H_2O_2-induced constriction to dilation in arterioles of db/db mice (Fig. 4, A and B). The TP receptor agonist U-46619-induced constrictions were not significantly different in the two groups of vessels, whereas
Growing evidence indicates that ROS act in the vasculature by modulating specific redox-sensitive signal transduction pathways and transcriptional regulatory events (25, 28). Oxidative stress, occurring in response to hyperglycemia (3, 38), hyperinsulinemia (11), hypertension (21), and Type 2 diabetes mellitus, is now considered to be one of the main mechanisms responsible for macro- and microvascular diseases (8). In this context, previously, we have demonstrated that, in db/db mice, a genetic model of Type 2 diabetes mellitus, vascular production of superoxide anion is elevated because of the increased vascular NAD(P)H oxidase activity, in association with the simultaneous reduction in vascular catalase activity (4, 5). In addition, recently, we and others have reported that, in these db/db mice, the vascular expression of COX-2 is upregulated (2, 15). Also, in clinical studies, it has been suggested that a low-grade vascular inflammation contributes to vascular diseases in patients with Type 2 diabetes mellitus (16, 42). The present study was designed to elucidate a possible interrelationship between oxidative stress and prostaglandin-mediated regulation of vascular tone in Type 2 diabetes mellitus. Specifically, we have tested the hypothesis that, in Type 2 diabetic mice, an enhanced production of vascular H$_2$O$_2$ interferes with the regulation of normal arteriolar tone via a prostaglandin-dependent mechanism.

Earlier studies already proposed a role for H$_2$O$_2$ in mediation of arteriolar responses even under physiological conditions (27, 41). It has been found that H$_2$O$_2$ elicited either arterial constriction (13, 32) or dilation (27, 29, 41), depending on the vessel type studied. Interestingly, in rat skeletal muscle arterioles, we have found that exogenously administered H$_2$O$_2$ elicited a biphasic effect on arteriolar diameter, causing constriction at lower and dilation at higher concentrations (9). In addition, there are recent studies suggesting that H$_2$O$_2$ contributes to the mediation of the pressure-induced diameter response (32). On the other hand, many more studies documented a pivotal role for H$_2$O$_2$ in the development of vascular dysfunction in pathological conditions, such as atherosclerosis, hypertension, and diabetes mellitus (20, 26, 35, 37). It has also been speculated that, in the vessel wall, H$_2$O$_2$-mediated mechanisms may compensate for the loss of NO-mediated dilation during the development of various diseases (8). In a recent study (13), H$_2$O$_2$ was implicated in the mediation of an enhanced, TxA$_2$/PGH$_2$-dependent contraction of rat aortic rings isolated from spontaneously hypertensive rats, suggesting that the role of H$_2$O$_2$-mediated mechanisms may be specific to certain diseases and/or vascular beds.

In the present study, first we confirmed our previous finding (2) that, in isolated arterioles from db/db mice, a greater basal tone develops in response to increases in intraluminal pressure than in those isolated from control mice (Fig. 1A). In addition, we found that removal of endothelium did not affect significantly the tone of arterioles in either group of vessels, suggesting that smooth muscle-dependent mechanisms are responsible for the enhanced tone in arterioles of db/db mice (Fig. 1B). To elucidate the possible role of H$_2$O$_2$ in pressure-induced responses, arterioles were studied in the presence of catalase, aiming to reduce the level of H$_2$O$_2$. Catalase had no effect on the tone of control arterioles at any pressure step investigated, whereas, in arterioles of db/db mice, catalase elicited an increase in diameter; hence, it reduced the level of arteriolar tone to the level of control at each pressure step (Fig. 1A).

The novel finding of the present study is that, in mice with Type 2 diabetes mellitus, there is an increased production of H$_2$O$_2$ in vascular smooth muscle cells, which leads to enhanced TxA$_2$/PGH$_2$-mediated basal tone of skeletal muscle arterioles.

**DISCUSSION**

**Detection of 3-nitrotyrosine by immunohistochemistry.** Immunohistochemical studies revealed an enhanced 3-nitrotyrosine staining in sections of gracilis muscle of db/db mice, which was located primarily in the inner layer of the arterioles (most likely endothelium and subendothelium) (Fig. 5A).

**Fig. 1.** Normalized diameter of pressure-induced responses of isolated arterioles from control (n = 7) and db/db (n = 7) mice in the absence and presence of catalase (A) and in endothelium-denuded (−endo) vessels (B). Data are means ± SE. *Significant differences (P < 0.05).

the U-46619-induced constrictions were completely inhibited with SQ-29548 (Fig. 4C).

**Fig. 5A.** Immunohistochemical staining of 3-nitrotyrosine in sections of gracilis muscle from control (A) and db/db (B) mice. The staining is found primarily in the inner layer of the arterioles (most likely the endothelium and subendothelium).
Next, we have demonstrated that, when compared with control vessels, formation of H$_2$O$_2$, indicated by H$_2$O$_2$-sensitive fluorescence labeling, is significantly elevated in carotid arteries of db/db mice and is then substantially reduced by catalase treatment (Fig. 2). Also, H$_2$O$_2$-sensitive fluorescence was negligible in control vessels. Although the carotid artery is a conduit blood vessel and findings obtained in carotid arteries cannot be directly extrapolated to microvessels, together with the functional results obtained with catalase in isolated arterioles, we propose that an elevated vascular level of H$_2$O$_2$ contributes to the enhanced tone of diabetic arterioles (documented in our previous and present studies). Our findings also indicate that H$_2$O$_2$ activates vascular mechanisms, which lead to constriction of skeletal muscle arterioles of db/db mice. Thus arteriolar responses were also obtained in response to exogenously administered H$_2$O$_2$. In isolated arterioles, we have used higher concentrations of H$_2$O$_2$ (10$^{-7}$ to 10$^{-4}$ mol/l), which are known to elicit dilations (9). Cumulative doses of H$_2$O$_2$ resulted in substantial dilations in both endothelium-intact and -denuded arterioles of control animals (Fig. 3). In contrast, in arterioles of db/db mice, H$_2$O$_2$ resulted in contractions, which were similar in magnitude regardless of whether or not the endothelium was present (Fig. 3). The results obtained in control vessels confirm previous observations showing that, depending on the vessel type, H$_2$O$_2$ elicits vasodilation via prostaglandins (39) or directly activating potassium channels in vascular smooth muscle cells (9, 29).

Our present finding in diabetic arterioles, however, indicated that H$_2$O$_2$-evoked constrictor mechanisms could override H$_2$O$_2$-induced dilation. Interestingly, in a recent study it was found that H$_2$O$_2$ caused TP receptor-mediated contraction of rat aortic rings isolated from spontaneously hypertensive rats.
Together with our previous findings showing that, in skeletal muscle arterioles of db/db mice, TxA2/PGH2 release enhances arteriolar tone (2), it was logical to hypothesize that, in skeletal muscle arterioles of db/db mice, an elevated level of H2O2 is involved in the enhanced production of constrictor prostaglandins. Indeed, we found that H2O2-induced constrictions were converted to dilations by the TP receptor antagonist SQ-29548 in arterioles of db/db mice (Fig. 4B). On the other hand, TP receptor agonist U-46619-induced constriction was similar in the two groups, showing that there is no difference in the sensitivity of TP receptors between arterioles of control and db/db mice (Fig. 4C). These data together suggest that, in diabetic arterioles, H2O2 induces TxA2/PGH2 release, which prevents the development of H2O2-mediated dilation.

The interaction between H2O2 and prostaglandin metabolism and regulation of arteriolar tone has been a subject of several previous investigations (13, 14, 17, 27, 35). It is known that prostanoids are synthesized from arachidonic acid catalyzed by phospholipase A2, cyclooxygenases (COX-1 and COX-2 enzymes), and specific downstream enzymes such as TxA2 synthase (7). In addition, vasoactive prostaglandins, such as isoprostanes, can be formed from arachidonic acid by free radical catalyzed peroxidation (34). Interestingly, it has been shown that isoprostanes can also activate TP receptors (23, 30); thus their role in H2O2-dependent TP receptor activation cannot be excluded in the present study.

In our previous study, H2O2-induced arteriolar constrictions were completely abolished either by the TP receptor antagonist SQ-29548 or by the COX inhibitor indomethacin (9, 29), similar to the findings by Leffler et al. (27). These data, together with the findings of the present study, suggest that increased activation of TP receptors are due to COX-derived metabolites, PGH2/TxA2. In this context, recently, we (2) and another group (15) have demonstrated that vascular expression of COX-2 of db/db mice is elevated. It has been also demonstrated that, in purified preparations of human COX-1 and COX-2, even when both are present in the same intracellular compartment, effective prostaglandin synthesis from arachidonic acid proceeds only through the COX-2 isoinform (24). On the basis of our previous and present findings, therefore, we propose that, in skeletal muscle arterioles of Type 2 diabetic mice, the vascular level of H2O2 is elevated, which induces constrictor prostaglandin, TxA2/PGH2, release, likely to be mediated by the upregulated COX-2 pathway (Fig. 5B). Our findings are in accordance with recent reports (1, 6, 33) showing that COX-2 expression is associated with enhanced production of constrictor prostaglandins, contributing to vascular disturbances under pathological conditions, such as diabetes mellitus.

It should be noted, however, that, in the present study, the TP receptor antagonist SQ-29548 did not completely reverse H2O2-induced arteriolar constrictions to dilations (Fig. 4B). Previous studies (39) revealed that H2O2 is able to induce dilator prostaglandin synthesis. In this context, we have earlier demonstrated that in gracilis muscle arterioles of db/db mice, administration of arachidonic acid elicited a reduced dilation, despite the presence of the TP receptor antagonist (2). These findings suggested that, in diabetic vessels, parallel to the enhanced constrictor prostaglandin production, synthesis of dilator prostaglandins is also impaired (2). One of the mechanisms responsible for the impaired dilator prostaglandin synthesis could be the inactivation of prostacyclin synthase by tyrosine nitration, as suggested previously by Zou et al. (44, 45). On the other hand, inactivation of prostacyclin synthase could be responsible for the increased synthesis of constrictor prostaglandins by facilitating the vascular production of the upstream constrictor prostaglandin PGH2 (46). To furnish evidence for this idea in diabetes mellitus, immunohistochemical studies

![Fig. 3. Original tracing (A) and summarized data (B) of arteriolar responses to exogenously administered H2O2 in skeletal muscle arterioles isolated from control (n = 5) and db/db (n = 5) mice before or after endothelium removal (C). Data are means ± SE. *Significant differences (P < 0.05).](http://ajpheart.physiology.org/)

H2O2-INDUCED ARTERIOLAR PG RELEASE IN TYPE 2 DIABETES

H653

AJP-Heart Circ Physiol • VOL 292 • JANUARY 2007 • www.ajpheart.org
were also performed to demonstrate an enhanced tyrosine nitration in arterioles of db/db mice. These studies revealed that when compared with control vessels, in arterioles of db/db mice there was an enhanced 3-nitrotyrosine staining, which was primarily localized in the inner layer of arterioles (most likely to the endothelium and subendothelium) (Fig. 5A). These findings suggest that tyrosine nitration, hence inactivation of prostacyclin synthase, may indeed occur in diabetic vessels. On the basis of previous and present findings, we propose that, in mice with Type 2 diabetes mellitus, due to the increased ROS production, in addition to the inactivation of prostacyclin synthase in endothelium, an increased level of H$_2$O$_2$ in the smooth muscle cells enhance constrictor prostaglandin synthesis leading to increased basal tone of skeletal muscle arterioles (Fig. 5B). More detailed mechanisms by which H$_2$O$_2$ upregulates COX-2 expression and activates the synthesis of constrict-

Fig. 4. Original tracing (A) and summarized data (B) of arteriolar responses to exogenously administered H$_2$O$_2$ in skeletal muscle arterioles isolated from control ($n = 5$) and db/db ($n = 5$) mice in the presence of the thromboxane A$_2$/prostaglandin H$_2$ (TP) receptor antagonist SQ-29548. C: arteriolar responses to U-46619 in skeletal muscle arterioles isolated from control ($n = 7$) and db/db ($n = 7$) mice in the absence and presence of the TP receptor antagonist SQ-29548. Data are means ± SE. *Significant differences ($P < 0.05$).

Fig. 5. A: representative images of immunohistochemical staining of 3-NT in gracilis muscle from control ($n = 3$) and db/db ($n = 3$) mice (scale bars, 50 μm). Arrows indicate the brown product of the diaminobenzidine tetrahydrochloride staining. B: potential mechanisms responsible for the increased production of constrictor prostaglandins, thromboxane A$_2$/prostaglandin H$_2$ (TxA$_2$/PGH$_2$), in the skeletal muscle arterioles in Type 2 diabetes mellitus. The enhanced production of NAD(P)H-oxidase-derived superoxide anion (4, 5), likely due to the increased levels of glucose in Type 2 diabetes, interacts with nitric oxide (NO) to form peroxynitrite (ONOO$^-$), which inactivates prostacyclin synthase in the endothelium (46), reducing dilator prostacyclin production and facilitating the release of constrictor PGH$_2$. In addition, our previous and present findings suggest a novel mechanism, according to which an elevated vascular level of H$_2$O$_2$ activates and/or upregulates cyclooxygenase-2 (COX-2) (2) in the smooth muscle cells leading to an increase in TxA$_2$/PGH$_2$ synthesis, which, by activating TP receptors, leads to enhanced tone of skeletal muscle arterioles. AA, arachidonic acid.
tor prostaglandins in Type 2 diabetes mellitus have yet to be elucidated in future studies. Nevertheless, the mechanisms revealed by the present study might have additional importance, since clinical studies have revealed that diabetic patients have hyperreactive platelets with exaggerated tendency for adhesion and aggregation (31), which is likely to be due to the increased TxA2 release from the arteriolar smooth muscle.

Taken together, our findings suggest that, in arterioles isolated from the skeletal muscle of db/db mice, the production of \( \text{H}_2\text{O}_2 \) is markedly enhanced, which contributes to the increased TxA2/PGH2-mediated basal tone. The link between oxidative stress and constrictor prostanoid synthesis—inferred in the present study—could be one of the initial steps in the pathological pathway leading to vascular dysfunction contributing to the disturbed regulation of tissue blood flow and atherothrombosis in Type 2 diabetes mellitus. Besides its effects on the basal arteriolar tone, an enhanced synthesis of TxA2/PGH2 in the vascular wall could provoke thrombosis by inducing platelet activation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Eniko T. Pasztor in the immunohistochemical experiments. Z. Bagi holds a Bolyai Fellowship.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants PO1-HL-43023 and HL-46813; American Heart Association Northeast GRANTS ACKNOWLEDGMENTS

REFERENCES


