Elevated Glucose Potentiates Contraction of Isolated Rat Resistance Arteries and Augments Protein Kinase C–Induced Intracellular Calcium Release

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The effect of elevated glucose on arterial contractions and intracellular calcium ($[Ca^{++}]_i$) release induced by protein kinase C (PKC) activation and potassium depolarization (KCl) was investigated. Mesenteric resistance arteries ($\phi < 200$ μm) isolated from male Wistar rats were studied using an arteriograph system that allowed control of transmural pressure (TMP) and measurement of lumen diameter. Arteries were incubated in either 11 or 44 mmol/L glucose and the concentration-response to Indolactam V (ILV; a specific PKC activator; LC Laboratories, Woburn, MA) and KCl was determined, as well as the sensitivity to Ca^{++} in the presence of either agonist. An additional group of arteries were incubated in 5.5 mmol/L glucose and the concentration-response to ILV was compared versus 11 and 44 mmol/L glucose. Arteries in 44 mmol/L glucose were more sensitive to both ILV and KCl, contracting to 10.0 μmol/L ILV, 53.9 ± 10.1% in 11 mmol/L versus 85.1 ± 2.0% in 44 mmol/L glucose ($P < .01$); arteries in 5.5 mmol/L glucose responded the least to ILV, contracting only 36.0 ± 4% to 10.0 μmol/L ILV ($P < .01$ *v* 11 and 44 mmol/L glucose). The KCl EC_{50} (ie, the value at which the agonist produced 50% maximal contraction) for 11 versus 44 mmol/L glucose was 41.3 ± 4.8 versus 31.1 ± 1.2 mmol/L ($P < .05$). There was no change in Ca^{++} sensitivity in elevated glucose for either agonist; however, Ca^{++} sensitivity was augmented threefold for ILV versus KCl, demonstrating an agonist-dependent modulation of Ca^{++} sensitivity. The Ca^{++} EC_{50} for ILV and KCl in 11 versus 44 mmol/L was 0.18 ± 0.05 versus 0.21 ± 0.05 and 0.59 ± 0.09 versus 0.60 ± 0.10 μmol/L ($P < .01$ *v* ILV). The effect of elevated glucose on $[Ca^{++}]_i$ release from the sarcoplasmic reticulum (SR) was investigated in arteries incubated in zero Ca^{++} buffer containing either 11 or 44 mmol/L glucose by measuring the contraction produced by 50 mmol/L caffeine, 3.0 μmol/L ILV, or 60 mmol/L KCl. Contraction to caffeine in 11 versus 44 mmol/L glucose was comparable, constricting 42.0 ± 6.0% in 11 mmol/L and 36.0 ± 4.4% in 44 mmol/L glucose ($P > .05$), and contraction to KCl was almost undetectable in both glucose concentrations. However, contraction to ILV increased from 5.6 ± 0.9% in 11 mmol/L to 18.7% ± 2.2% in 44 mmol/L glucose ($P < .01$), indicating that although the amount of Ca^{++} in the SR (caffeine-sensitive stores) was not increased in elevated glucose, PKC-induced release of $[Ca^{++}]_i$ was enhanced, a consequence that may explain the noted glucose-induced increase in contraction to PKC activation.

HYPERGLYCEMIA is thought to be a major contributor to the vascular complications of diabetes mellitus[1-3]; however, the cellular mechanisms by which elevated glucose exerts effects on vascular contractile function are unclear. Intracellular mediators of contraction, including diacylglycerol (DAG) and protein kinase C (PKC), have been shown to be increased in diabetic animal models and in cultured vascular cells exposed to high glucose.[4-12] Glucose-induced increases of DAG in vascular smooth muscle have been shown to be due to an increased flux of glucose through the glycolytic pathway, augmenting de novo synthesis of DAG.[4] Endogenous 1,2-diacyl-*sn*-glycerol (DAG) is the primary activator of PKC,[13,14] and its accumulation in response to elevated glucose has been proposed as a mechanism for enhancing membranous PKC activity, including translocation of the kinase molecule from the cytosol to the membrane.[4,6]

PKC is a ubiquitous intracellular signaling molecule involved in a wide variety of cellular responses.[13] In vascular smooth muscle, it is an integral component of several signal transduction cascades that regulate agonist-induced contraction and mechanotransduction.[13-16] It has also been shown to affect the sensitivity of the contractile apparatus to calcium, and is considered an important mediator of vascular tone by promoting the release of intracellular calcium ($[Ca^{++}]_i$) from the sarcoplasmic reticulum (SR).[13-16] Given the widespread role of PKC in modulating vascular tone and reactivity, its increased activity under conditions of elevated glucose could significantly affect arterial function.

This study used isolated rat mesenteric resistance arteries under pressurized conditions in vitro to investigate the effect of elevated glucose on mediators of arterial contraction, including PKC activation and potassium depolarization (KCl). Since these compounds elicit contraction by movement of calcium from either the extracellular fluid (KCl) or intracellular SR stores (PKC), we investigated the glucose-induced modulation of calcium sensitivity and SR calcium release that may affect the regulation of contraction under conditions of elevated glucose.

MATERIALS AND METHODS

Vessel Preparation and Pressurized Arteriograph System

Mesenteric arteries (inner diameter, 200 to 250 μm) were obtained from adult (aged 16 to 18 weeks) male Wistar rats (N = 39). The animals were anesthetized with ether and quickly decapitated as approved by the Oregon Health Sciences University institutional animal care department. The mesenteric arcade was carefully removed and placed in a dissecting dish filled with cold (4°C) oxygenated physiologic saline solution (PSS). Two third-order mesenteric arteries (the branch just prior to the perforating arteries) were carefully dissected and cleared of extraneous connective tissue. The isolated arteries were placed in the arteriograph chambers, also filled with oxygenated PSS.

The arteriograph (Living Systems, Burlington, VT) consisted of two 20-mL chambers with inlet and outlet ports to allow for superfusion of the vessels with PSS. The superfusate (PSS) was continually recirculated in a 50-mL reservoir and aerated with a gas mixture of 10% O_2, 5% CO_2, and 85% N_2, allowing pH adjustment to 7.4 ± 0.5 by increasing or decreasing the amount of gas bubbled From the reservoir,

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the superfusate was pumped through a heat exchanger and warmed to 37°C before entering the arteriograph chamber.

Each vessel chamber contained a proximal and distal glass microcannula (tip, 50 μm) on which the arteries were mounted and secured with a small strand (20-μm diameter) of nylon suture and perfused with oxygenated PSS. The proximal cannula was attached to a servomechanism system consisting of an in-line pressure transducer, a controller, and a small peristaltic pump that continually measured and adjusted the transmural pressure (TMP). In these experiments, all vessels were maintained at a constant TMP of 50 mm Hg.

The vessel chambers were mounted on an inverted microscope with an attached videocamera and monitor. The transilluminated image of the vessel on a videomonitor was used to electronically determine the dimensions (diameter and wall thickness) of the artery as previously described.[16] Briefly, the vessel image was aligned perpendicular to the video scan lines on the videomonitor. The optical contrast of the vessel wall produced voltage fluctuations in the video signal, which were used to initiate and terminate a voltage ramp, the amplitude of which is proportional to the diameter.[17] The output (analog voltage) was simultaneously displayed as a digital readout and sent to an IBM-compatible 386 computer interfaced with a serial data acquisition system (DATAQ Instruments, Akron, OH). A real-time waveform of the diameter and TMP was displayed on a VGA monitor, allowing for recording of dynamic responses, similar to a chart recorder.

Agents and Solutions

For the experiments involving concentration-response curves in two different glucose concentrations, the ionic composition of the PSS that both superfused and perfused the vessels was as follows (mmol/L): NaCl 119.0, $NaHCO_3$ 24.0, KCl 4.7, KH_2PO_4 1.18, $MgSO_4 \cdot 7H_2O$ 1.17, $CaCl_2$ 1.6, EDTA 0.026, and glucose 11.0 or 44.0. For experiments that used zero-calcium PSS, the ionic composition was the same except that no $CaCl_2$ was added. PKC activation was accomplished using (−)-Indolactam V (ILV; LC Laboratories, Woburn, MA), a synthetic alkaloidal activator of PKC. ILV binds to the same region on the PKC molecule as phorbol esters,[18] which mimic DAG in the activation of PKC and induce a slow sustained contraction in vascular smooth muscle.[16,19] All salts and glucose were purchased from VWR Scientific (Philadelphia, PA); prazosin (PZ), norepinephrine (NE), and caffeine were purchased from Sigma (St Louis, MO). PSS was made weekly and stored without glucose at 4°C. The appropriate amount of glucose was added to the PSS prior to each experiment. Stock solutions of KCl, $CaCl_2$, ILV, and caffeine were made weekly and also stored at 4°C. PZ and NE were made fresh daily.

Experimental Protocols

Effect of elevated glucose on contraction to KCl and ILV. Following a 60-minute equilibration in 11-mmol/L glucose PSS at TMP 50 mm Hg, PZ (1.0 μmol/L) was administered to block NE release resulting from KCl-induced depolarization. KCl was then cumulatively added to the reservoir to obtain concentrations of 10, 20, 40, 60, and 80 mmol/L, and the diameter at each concentration was recorded after reaching a stable contraction, at approximately 5 minutes. The vessel was then washed with 11-mmol/L glucose PSS, and an ILV concentration-response curve (0.1 to 10 μmol/L) was generated by cumulatively adding ILV to the bath. Again, the diameter was recorded at each concentration of agonist after a stable contraction was reached, at approximately 10 minutes. Once both concentration-response curves were completed, 11-mmol/L glucose PSS was washed out and replaced with 44-mmol/L glucose PSS. The vessel was then incubated in high glucose for 2 hours, and the concentration-response to both KCl and ILV was repeated. At the end of each experiment, a maximal dose of NE (10.0 μmol/L) was administered and the diameter recorded.

Effect of elevated glucose on calcium sensitivity in the presence of ILV or KCl. To test the calcium sensitivity of the two agonists in different glucose concentrations, arteries (n = 13) were dissected in a zero-calcium PSS containing 11 mmol/L glucose. This same solution was used as the perfusate and superfusate during equilibration at 50 mm Hg. Following equilibration, either KCl (60.0 mmol/L) or ILV (3.0 μmol/L) were added to the bath. $CaCl_2$ (0.01 to 3.0 mmol/L) was then cumulatively added to the PSS, and the arterial diameter at each level of calcium was measured. After the calcium concentration-response was generated separately for both agonists, 11-mmol/L glucose PSS was replaced with 44-mmol/L glucose PSS and the vessels were incubated for 2 hours, after which the calcium concentration-response was repeated with both agonists.

Effect of elevated glucose on $[Ca^{++}]_i$ store and release. To investigate the effect of elevated glucose levels on $[Ca^{++}]_i$ stores (ie, SR calcium), the magnitude of contraction to agonists known to release SR calcium was measured in zero-calcium buffer in both glucose concentrations. After equilibration in zero-calcium buffer containing 11 mmol/L glucose, one of the following agonists was added and the diameter recorded: 50 mmol/L caffeine (n = 6), 3.0 μmol/L ILV (n = 7), or 60.0 mmol/L KCl (n = 6). Caffeine is known to discharge calcium from caffeine-sensitive stores in the SR, resulting in a transient constriction, the magnitude of which is considered a measure of total SR calcium.[20] Activation of PKC with ILV selectively opens SR calcium channels, and therefore, contraction to this compound in zero-calcium buffer is used to determine the effects on SR calcium release.[19,21] Since KCl causes contraction by depolarization of the plasma membrane, with an influx of extracellular calcium, this compound should have little effect in zero calcium and was used as a control.[22] After measurement of contraction in 11 mmol/L glucose, the vessels were washed with zero-calcium PSS containing 44 mmol/L glucose and incubated for 2 hours, after which the response to the agonists was determined.

Control vessels. To determine that any effect observed was due to the vessel responding in high-glucose conditions and not to an effect of performing two concentration-response curves consecutively, the second chamber of the arteriograph was used to analyze control vessels (n = 13). These vessels were kept in 11-mmol/L glucose PSS for the entire experiment instead of increasing the glucose level for the second set of concentration-response curves in 44 mmol/L glucose as described earlier.

To investigate the dependence of the contraction in response to PKC activation on the glucose concentration, a separate group of arteries (n = 3) were incubated in a low concentration of glucose (5.5 mmol/L) and the concentration-response to ILV was determined as described earlier.

Data Calculations

The concentration-response for each artery was normalized to the diameter invoked by a maximal dose of NE (10.0 μmol/L). The value at each concentration of agonist was determined by the equation, $[(\phi_{start} - \phi_{dose})/(\phi_{start} - \phi_{NE})] \cdot 100\%$, where ϕ_{start} is the diameter at the start of the experiment, ϕ_{dose} is the diameter invoked by the concentration of agonist, and ϕ_{NE} is the diameter produced by 10.0 μmol/L NE.

EC_{50} values for KCl and calcium were determined by normalizing the data to the maximal response and plotting the concentration-response on a logarithmic scale. The EC_{50} value was extrapolated from a best-fit line between 20% and 80% of the maximal contraction by determining the value at which the agonist produced 50% of the maximal contraction (ie, EC_{50}). Contraction produced by ILV, KCl, and caffeine in zero-calcium PSS was determined as a percent decrease in diameter by the equation, $[1 - (\phi_{agonist}/\phi_{start})] \cdot 100\%$, where $\phi_{agonist}$ is the diameter produced by addition of the agonist and ϕ_{start} is the diameter at the start of the experiment.

Statistical Analysis

The results are expressed as the mean ± SE. To determine differences in the contractile response of vessels to ILV versus KCl, calcium sensitivity in the presence of the two agonists, and contraction produced by the agonists in zero-calcium PSS in 11 versus 44 mmol/L glucose, ANOVA with repeated measures was used and considered significant at a P level .05. Differences in the contractile response to ILV in different glucose concentrations were determined using ANOVA with three treatment groups, considered significant at a P level .05.

RESULTS

Contractile Response of Vessels to ILV and KCl in 11 and 44 mmol/L Glucose

Incubation of the arteries in any of the glucose concentrations had no effect on resting diameter. Addition of the PKC agonist ILV to the arterial bath caused a concentration-dependent contraction in 11 mmol/L glucose, which was significantly enhanced after incubation in 44-mmol/L glucose PSS at each concentration of ILV studied. The response of vessels to the maximal concentration of ILV (10.0 μmol/L) increased from 53.9 ± 10.1% in 11 mmol/L glucose to 85.1 ± 2.0% in 44 mmol/L glucose ($P < .01$). The concentration-response to ILV in 11 versus 44 mmol/L glucose is shown in Fig 1. Control vessels, in which the concentration-response to ILV was repeated in 11 mmol/L glucose instead of 44 mmol/L glucose, responded similarly each time, producing contractions to 10.0 μmol/L ILV of 57.0% ± 35.0% for the first response in 11 mmol/L glucose and 67.0% ± 16.0% for the second response in 11 mmol/L glucose ($P > .05$, data not shown). Arteries incubated in 5.5 mmol/L glucose produced the lowest amount of contraction at each concentration of ILV (Fig 1). The dependence on the glucose concentration of the contraction to ILV is shown in Fig 2.

Contraction elicited by KCl was also enhanced in the high-glucose PSS. The EC_{50} value for arteries in 11 versus 44 mmol/L glucose was 41.3 ± 4.8 versus 31.1 ± 1.2 mmol/L ($P < .05$). The response of the arteries increased from 55.1 ± 8.6% in 11 mmol/L to 81.6 ± 4.6% in 44 mmol/L glucose ($P < .05$). In addition, the first concentration of KCl (10 mmol/L), which did not produce any response in 11-mmol/L glucose PSS, dilated the vessels after incubation in 44-mol/L glucose PSS, indicating a glucose-induced depolarization. The concentration-response curve to KCl in 11 and 44 mmol/L glucose is shown in Fig 3. Control vessels, in which KCl responses were repeated in 11 mmol/L glucose, responded comparably each time, with an EC_{50} of 41.0 ± 2.0 mmol/L for the first response versus 45.0 ± 2.0 mmol/L ($P > .05$, data not shown).

Contractile Response to Calcium in the Presence of ILV or KCl in 11 and 44 mmol/L Glucose

The addition of $CaCl_2$ to the arteries produced a concentration-dependent contraction in vessels containing either ILV (3.0 μmol/L) or KCl (60.0 mmol/L) in zero-calcium PSS. There was no difference in calcium sensitivity between 11 and 44 mmol/L glucose when either ILV (EC_{50}, 0.18 ± 0.05 mmol/L in 11 mmol/L glucose v 0.21 ± 0.05 in 44 mmol/L glucose, $P > .05$) or KCl (EC_{50}, 0.59 ± 0.09 in 11 mmol/L v 0.60 ± 0.10 in 44

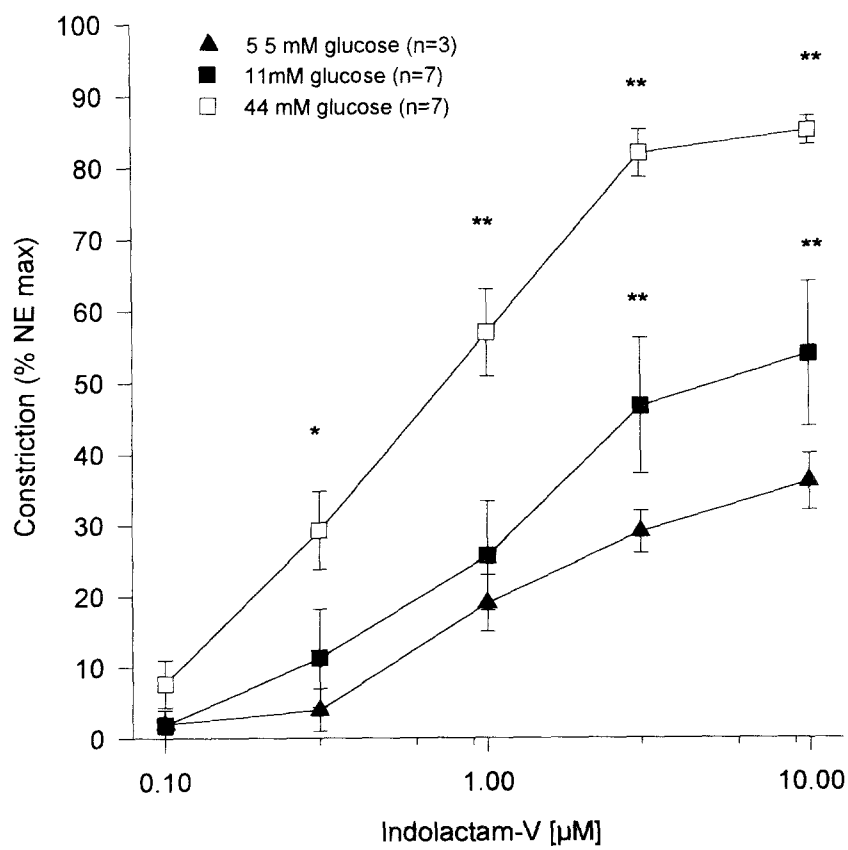

Fig 1. Concentration-response to PKC agonist ILV in 5.5 mmol/L, 11 mmol/L, and 44 mmol/L glucose. Data are normalized to the constriction produced by a maximum concentration of NE (10 μmol/L). *$P < .05$, **$P < .01$.

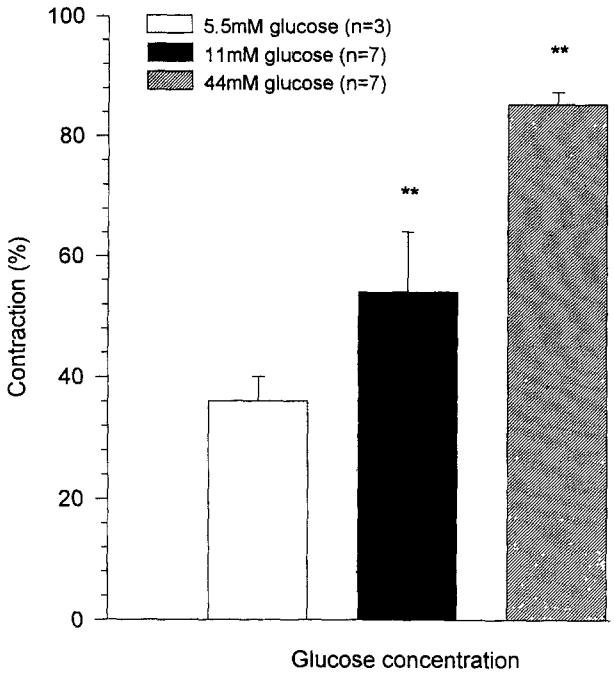

Fig 2. Contraction produced by 10.0 μmol/L ILV in arteries incubated in 5.5 mmol/L, 11 mmol/L, and 44 mmol/L glucose. **$P < .01$.

mmol/L glucose, $P > .05$) was used as the constrictor agent. However, the calcium sensitivity of arteries in both glucose concentrations was significantly increased when contraction was elicited by ILV versus KCl, indicating an agonist-induced modulation of calcium sensitivity. The concentration-response to calcium in the presence of ILV and KCl in 11 and 44 mmol/L glucose is shown in Fig 4.

Effect of Elevated Glucose on Contraction to $[Ca^{++}]_i$ Release

The addition of 3.0 μmol/L ILV to zero-calcium PSS containing 11 mmol/L glucose elicited a contraction of 5.6 ± 0.9%, which increased to 18.7 ± 2.2% in 44-mmol/L glucose PSS ($P < .01$). Vessels exposed to KCl (60 mmol/L) instead of ILV produced contractions in zero-calcium PSS that were nearly undetectable in either glucose concentration, reducing the lumen diameter 2.7 ± 1.9% in 1-mmol/L glucose PSS and 0.7 ± 0.3% in 44-mmol/L glucose PSS ($P > .05$). Exposure of the arteries in zero-calcium PSS to caffeine produced a transient decrease in diameter in arteries exposed to 11 mmol/L glucose, which was not significantly different in arteries exposed to 44-mmol/L glucose PSS. The amplitude of the contraction produced by 50 mmol/L caffeine was 42.0 ± 6.0% in 11 mmol/L glucose and 36.0% ± 4.4% in 44 mmol/L glucose ($P > .05$). The percent decrease in diameter elicited by each agonist, compared with 50 mmol/L caffeine, in zero-calcium PSS containing 11 versus 44 mmol/L glucose is shown in Fig 5.

DISCUSSION

Elevated levels of glucose have been shown to alter the metabolism of vascular smooth muscle cells in culture.[4,5] The subsequent increased utilization of glucose produces an excessive amount of DAG, affecting intracellular signaling by augmenting activation of PKC.[4,6-8,12,23] In the present study, we have established that a functional consequence of these glucose-induced alterations is a potentiation of constrictor responses in

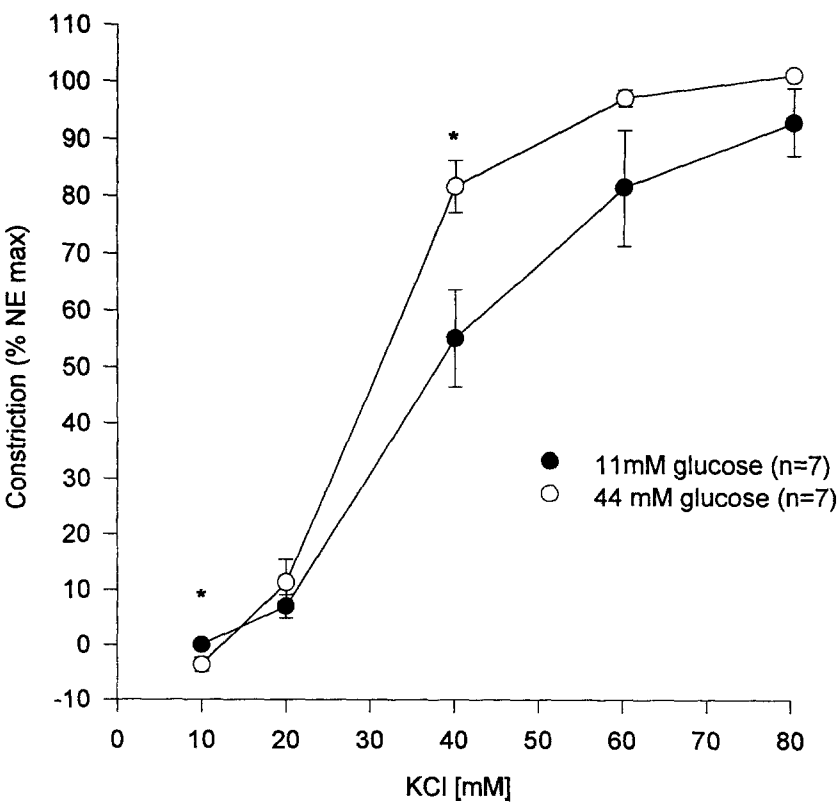

Fig 3. Concentration-response to KCl-induced depolarization in 11 mmol/L and 44 mmol/L glucose. Data are normalized to the constriction produced by a maximum concentration of NE (10 μmol/L). *$P < .05$.

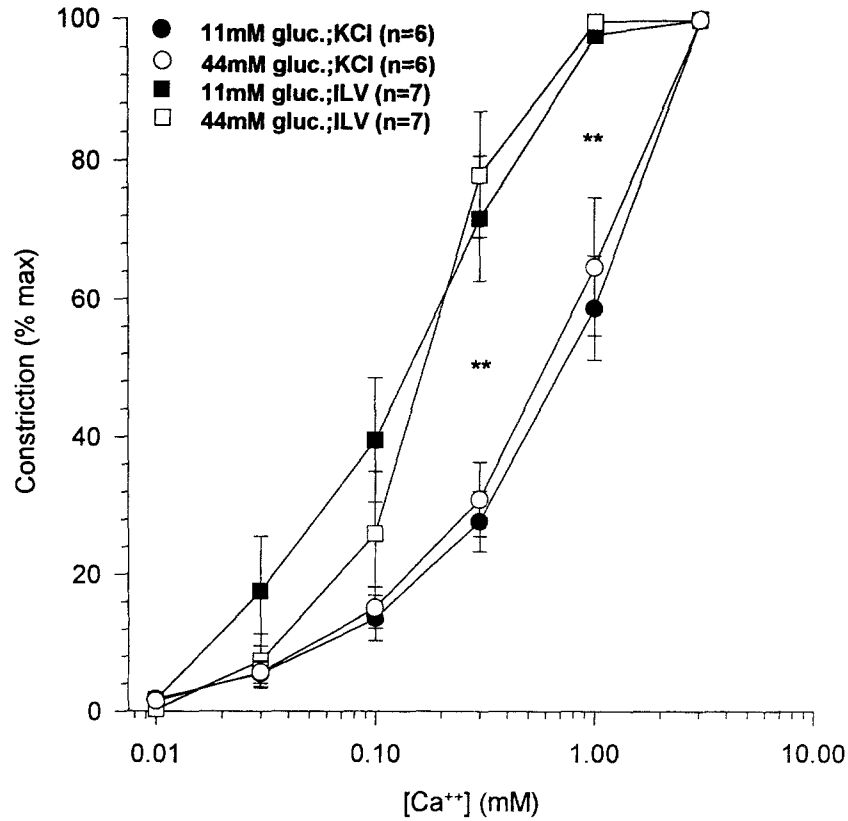

Fig 4. Concentration-response to calcium in the presence of either 60.0 mmol/L KCl or 3.0 μmol/L ILV in 11 mmol/L and 44 mmol/L glucose. Data are normalized to the constriction produced by a maximum concentration of NE (10 μmol/L). **$P < .01$.

resistance arteries to both PKC activation and KCl depolarization. In addition, augmented PKC-induced contraction in high glucose was associated with increased SR calcium release. Given the widespread role of calcium as a signaling molecule, altered calcium handling within vascular smooth muscle could affect many cellular processes including contraction, growth, and secretion during periods of elevated glucose.

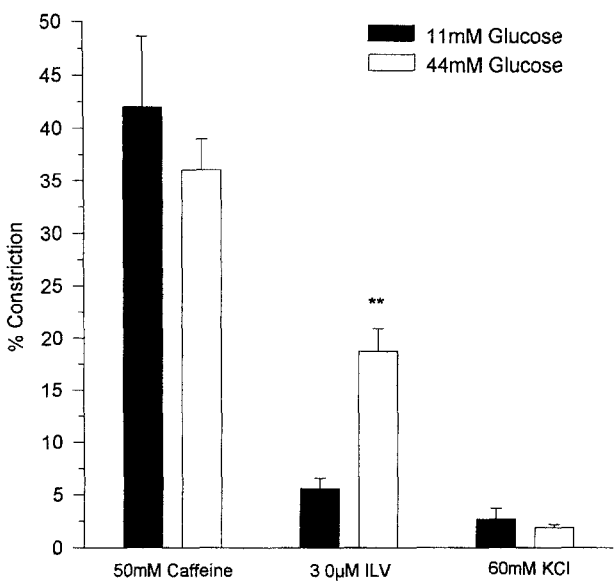

Fig 5. Percent constriction elicited by 50 mmol/L caffeine, 3.0 μmol/L ILV, and 60.0 mmol/L KCl in zero-calcium buffer containing either 11 mmol/L or 44 mmol/L glucose. **$P < .01$.

The present study demonstrated that contraction to PKC activation by ILV was enhanced in arteries incubated in elevated glucose. While initial experiments demonstrated an increased PKC-induced contraction in 44 versus 11 mmol/L glucose, further experiments in 5.5 mmol/L glucose showed that this lower concentration produced only a modest contraction to ILV compared with arteries in higher concentrations of glucose (Fig 2). These results demonstrate that the PKC-induced contraction was glucose-dependent, such that increased concentrations of glucose produced increased contraction to ILV.

The contraction to KCl depolarization was also enhanced in elevated glucose (Fig 3). Contraction of vascular smooth muscle is regulated primarily by the concentration of $[Ca^{++}]_i$ and activated by the movement of calcium into the cytosol from two sources, the extracellular fluid and SR.[20,24,25] Movement of calcium ions across the sarcolemmal and SR membranes is mediated through the action of several membrane pumps and ion channels.[24] Therefore, glucose-induced augmentation of contraction could be accomplished by (1) an increased influx of calcium into the cytosol from either the extracellular fluid or SR, (2) a decrease in calcium efflux to the extracellular fluid or SR, or (3) an increase in calcium sensitivity of the smooth muscle contractile apparatus such that less calcium is needed to produce a similar degree of force.

The agonists used in this study cause vascular smooth muscle to contract by affecting different mechanisms of calcium influx. KCl causes depolarization of the plasma membrane, opening

voltage-operated calcium channels and increasing $[Ca^{++}]_i$ through flux from the extracellular fluid.[22] This was confirmed in arteries in zero-calcium PSS that barely responded to KCl addition, since an extracellular calcium gradient is necessary for contraction to KCl (Fig 5). In contrast, the mechanism by which ILV causes contraction is PKC activation, which has several effects on smooth muscle.[13-16,21] PKC binds to and opens calcium channels in both the plasma membrane and SR and can therefore cause contraction by affecting calcium flux from either the extracellular fluid or SR.[13,21] Since arteries contracted to ILV in zero-calcium PSS (Fig 5), the contraction to PKC activation was mediated in part by the release of calcium from the SR.

ILV is a specific agonist for PKC that binds to the same regulatory subunit of the PKC molecule as DAG.[16,18] Activation of PKC involves binding DAG (or ILV), phospholipid, and calcium. Phospholipid and calcium act synergistically to promote localization of PKC to the membrane where DAG binds and activates PKC.[13] The sensitization to ILV-induced contraction demonstrated in high glucose may be due to already-elevated DAG levels such that less agonist is necessary to promote contraction. Increased de novo synthesis of DAG in response to elevated glucose has been demonstrated in vascular smooth muscle.[4] Alternatively, the calcium-dependence of PKC activation may provide a positive-feedback mechanism for enhancing PKC activity and contraction. While measuring PKC and its subcellular distribution would be helpful for understanding the specific activity of this molecule in these arteries, it is beyond the scope of this study, which has demonstrated increased PKC-induced contraction with a concomitant increase in SR calcium release in response to elevated glucose.

Elevated glucose caused a 35% increase in contraction to 3.0 μmol/L ILV in the presence of extracellular calcium and a 13% increase without calcium, indicating that a significant portion of the augmented contraction was due to enhanced $[Ca^{++}]_i$ release. Calcium release from the SR is accomplished by opening calcium channels within the SR membrane, allowing calcium ions to move down a concentration gradient.[25] In the present study, caffeine was used to unload calcium from the SR, the amplitude of which was used as a measure of the total amount of calcium in the SR.[20,26] Although these caffeine-induced contractions were not different between glucose concentrations, the contraction to ILV in zero-calcium PSS was increased in high glucose. indicating that while calcium influx into the SR was not affected by elevated glucose, specific release of SR calcium was augmented, which may contribute to the increased agonist-induced contraction.

Calcium can be extruded from the SR by inositol 1,4,5-triphosphate (IP_3), a diffusible second messenger produced after agonist stimulation by phosphoinositide turnover and activation of the phosphoinositide-specific phospholipase C pathway.[27-29] IP_3 interacts with a specific receptor on the SR membrane, which is also a calcium channel; binding opens the channel to release calcium into the cytosol.[26,30] Increased IP_3 levels could therefore explain the augmented contraction to ILV in high-glucose zero-calcium PSS. However, it seems unlikely that elevated IP_3 levels would be produced during periods of high glucose, since increased DAG is presumably derived from the glycolytic intermediates dihydroxyacetone and glycerol-3-phosphate generated from enhanced flux of glucose through the glycolytic pathway,[4,23] not from phospholipid turnover that would produce IP_3. Also, Lee et al[10] measured IP_3 in cultured vascular cells in response to elevated glucose and found the levels to be unchanged. However, glucose-induced PKC activation may cause the SR IP_3-receptor/calcium-release channel to induce calcium entry into the cytosol from the SR upon activation, since the channel is a proposed substrate for PKC.[31]

An alternative hypothesis for the augmented contraction in high glucose to PKC activation in zero-calcium PSS is a net decrease in calcium efflux from the cytosol by the Na^+-Ca^{++} exchanger. The Na^+-Ca^{++} exchanger is coupled to the sarcolemmal (Na,K)-adenosine triphosphatase (ATPase) or Na^+ pump, which is present in the plasma membrane of all eukaryotic cells and is a major regulator of the intracellular ionic environment.[24,32,33] Inhibition of the (Na,K)-ATPase pump by ouabain has been shown to increase $[Ca^{++}]_i$,[34] and is hypothesized to have a significant role in the modulation of vascular reactivity and regulation of blood pressure and to influence vascular tone.[35-37] A decrease in (Na,K)-ATPase activity has been found in a number of vascular tissues from diabetic animals and in response to elevated glucose in vascular smooth muscle cells.[9,10] A study by Ohara et al[38] found decreased (Na,K)-ATPase activity with a concomitant increase in $[Ca^{++}]_i$ in cultured aortic smooth muscle cells from diabetic rats, indicating a link between altered Na^+ pump activity and transmembrane flux of calcium ions in diabetic vessels.

PKC has been shown to affect several membrane pumps and ion channels known to be involved in sustaining membrane potential and ionic gradients, including (Na,K)-ATPase.[39,40] The catalytic α-subunit of (Na,K)-ATPase is a known substrate for PKC and the phosphorylation of this site interferes with pump activity.[40] The resulting decreased activity could cause diminished extrusion of Na^+ from the cytosol with a concomitant decreased efflux of calcium, resulting in both depolarization of the cell membrane and increased $[Ca^{++}]_i$.[39,40] The potentiated contraction to KCl-induced depolarization in elevated glucose (Fig 3) could result from an indirect effect of PKC interfering with (Na,K)-ATPase activity and inducing depolarization of the sarcolemma, thereby increasing $[Ca^{++}]_i$.

The effect of glucose on PKC-induced contraction could be mediated alternatively by an effect on the sensitivity of the contractile apparatus to calcium. PKC exhibits broad substrate specificity and has been shown to affect contractility via phosphorylation of contractile proteins (eg, myosin,[42] caldesmon,[43] and calponin[44]), acting to increase calcium sensitivity (ie, the calcium to force ratio is decreased).[24,45] Although there was no glucose-induced increase in calcium sensitivity with either agonist, the calcium sensitivity for PKC activation by ILV was three times greater than for KCl, demonstrating a PKC-induced enhanced responsiveness of smooth muscle to calcium (Fig 4). However, this result reflects the response of intact arteries to extracellular calcium, and a permeabilized preparation in which $[Ca^{++}]_i$ could be controlled would be more suitable for determining glucose-induced alterations of the calcium to force ratio.[45]

There are other ATP-dependent processes that may be

affected by an increased flux of glucose through the glycolytic pathway, including ion channels that control $[Ca^{++}]_i$.[46,47] For example, the Ca^{++}-ATPase present in the SR and plasma membranes controls calcium efflux and has been linked to metabolism and PKC activation.[46-48] However, an increase in its activity would cause a decrease in $[Ca^{++}]_i$ and promote relaxation. Similarly, increased activity of (Na,K)-ATPase would promote hyperpolarization and relaxation of the smooth muscle, not the increased contraction noted here. Although the limitations of the present study include the fact that PKC activity and ATP levels were not measured, it is fairly well established that augmented DAG and PKC levels in vascular smooth muscle occur in response to an increased flux of glucose through the glycolytic pathway.[4-7,23] Therefore, it seems likely that the increased PKC-induced contraction and possibly the enhanced KCl-induced contraction noted in the present study are due to an effect, either direct or indirect, of enhanced production of this important signaling molecule during periods of elevated glucose.

In summary, we have demonstrated that elevated glucose significantly augments contraction induced by PKC activation and KCl depolarization in mesenteric resistance arteries. Elevated glucose also appears to augment PKC-induced release of $[Ca^{++}]_i$ stores, which may have a major role in enhancing vasoconstriction. The augmented contraction in elevated glucose does not appear to be due to enhanced calcium sensitivity, but to an effect on $[Ca^{++}]_i$ handling influencing transmembrane calcium movement, leading to increased $[Ca^{++}]_i$.

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