Dilation of Retinal Arterioles in Response to Lactate: Role of Nitric Oxide, Guanylyl Cyclase, and ATP-Sensitive Potassium Channels

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Purpose. Lactate, a key metabolite in the retinal tissue, has been implicated in regulating retinal blood flow to match retinal metabolic demand. However, the direct effect of lactate on retinal vascular tone and the possible underlying signaling mechanisms remain unknown. In the present study, the roles of endothelium-derived vasodilators, guanylyl cyclase, and potassium channels were examined in lactate-induced dilation of retinal arterioles in vitro.

Methods. Porcine second-order retinal arterioles were isolated, cannulated, and pressurized to 55 cm H_2O lumenal pressure without flow. Diameter changes in response to agonists were recorded with videomicroscopic techniques.

Results. All vessels developed basal tone (~70 μm in internal diameter) and dilated dose dependently in response to neutralized L-lactate (0.01–10 mM). Inhibition of cyclooxygenase by indomethacin only slightly reduced the vasodilatory response to lactate. In contrast, blockade of monocarboxylate transporters, nitric oxide (NO) synthase, soluble guanylyl cyclase, and ATP-sensitive potassium (K_{ATP}) channels nearly abolished lactate-induced vasodilation. The cGMP phosphodiesterase inhibitor zaprinast enhanced the vasodilation response to lactate. Similar to the lactate-induced response, the vasodilation elicited by *S*-nitroso-*N*-acetylpenicillamine, an NO donor that activates cGMP signaling, was also inhibited by the soluble guanylyl cyclase and K_{ATP} channel blockers.

Conclusions. These data suggest that uptake of lactate by vascular cells via monocarboxylate transporters causes retinal arteriolar dilation predominantly via stimulation of NO synthase and subsequent activation of guanylyl cyclase. The guanylyl cyclase/cGMP signaling triggers opening of K_{ATP} channels for vasodilation. A better understanding of the fundamental signaling pathways responsible for lactate-induced dilation of retinal arterioles may help shed light on the possible mechanisms contributing to the metabolic regulation of retinal blood flow under physiological and pathophysiological conditions. (*Invest Ophthalmol Vis Sci.* 2006;47:693–699) DOI:10.1167/iovs.05-1224

Lactate is the major metabolite produced by anaerobic glycolysis in most tissues. However, in the retina, lactate is produced under aerobic and anaerobic conditions.[1,2] The main cellular sources of lactate in retinal tissue are the Müller glial and neuronal cells.[2–5] The released lactate has been suggested to act as a coupling mechanism between metabolic activity/demand and blood flow regulation in the retinal tissue.[6] Because the retinal circulation lacks autonomic innervation,[7] modulation of retinal vascular tone is fundamentally dependent on local control mechanisms, such as metabolic regulation.[8] The predominant site of local blood flow regulation is in the microvascular network, particularly the arteriolar vessels. Recent animal[9] and human[10] studies have demonstrated that intravenous administration of lactate can increase retinal blood flow under normal physiological conditions. In addition, exposure of retinal tissue to acute hypoxia causes compensatory dilation of retinal arterioles and an increase in lactate production.[11,12] Although evidence has shown that intravitreal administration of a high concentration of L-lactate (500 mM) causes dilation of retinal vessels in vivo,[6] the vasomotor effect of L-lactate, independent of retinal glial and neuronal tissue, on small retinal arterioles has not been determined.

Experimental evidence in large[13–15] and small arteries[16,17] from various vascular beds, other than retina, has demonstrated that L-lactate causes vasodilation in vitro. Several different mechanisms have been suggested to mediate this vasodilatory response. The activation of vascular smooth muscle guanylyl cyclase, independent of endothelium-derived nitric oxide (NO), appears to mediate the dilation of bovine pulmonary arteries,[13] rat skeletal muscle arterioles,[16] and human placental arteries.[14] In contrast, the opening of smooth muscle calcium–activated potassium (K_{Ca}) channels is involved in the dilation of porcine coronary arteries to lactate.[18] However, the underlying signaling mechanisms responsible for the vasomotor action of lactate in retinal arterioles remain unknown. To address these questions directly without confounding influences from metabolic, hemodynamic, humoral, and glial/neuronal factors associated with in vivo preparations, we isolated and pressurized porcine retinal arterioles without flow for in vitro study. We examined the vasomotor action of neutralized L-lactate and elucidated the relative roles of endothelium-derived vasodilators, guanylyl cyclase, and potassium channels in the vasomotor response of these microvessels.

Methods

Animal Preparation

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Scott and White Institutional Animal Care and Use Committee. Pigs (8–12 weeks old of either sex; 7–10 kg) purchased from Barfield Farms (Rogers, TX) were sedated with ketamine (4.4 mg/kg, intramuscularly) and xylazine (2.2 mg/kg, intramuscularly), anesthetized with sodium pentobarbital (30 mg/kg, intravenously), intubated, and ventilated with room air. Heparin (1000 U/kg) was administered into the marginal ear vein to prevent clotting, and the eyes were enucleated and immediately placed in a moist chamber on ice.

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Isolation and Cannulation of Microvessels

The anterior segment and vitreous body were removed carefully under a dissection microscope. The posterior segment or eye cup was placed in a cooled dissection chamber (~8°C) containing a physiological salt solution (PSS; in mM: NaCl 145.0, KCl 4.7, $CaCl_2$ 1.5, $MgSO_4$ 1.17, NaH_2PO_4 1.2, glucose 5.0, pyruvate 2.0, EDTA 0.02, and 3-(*N*-morpholino)propanesulfonic acid [MOPS] 3.0) with 0.1% albumin (USB, Cleveland, OH). Single second-order retinal arterioles (in the range of 40–60 μm in internal diameter in situ, 0.6–1.0 mm in length) were carefully dissected with a pair of Dumont microdissection forceps (Fine Science Tools, Foster City, CA) with the aid of a stereomicroscope (model SZX12; Olympus, Melville, NY). After careful removal of any remaining neural and connective tissues, the arteriole was then transferred for cannulation to a Lucite vessel chamber containing PSS-albumin solution equilibrated with room air at ambient temperature. One end of the arteriole was cannulated with a glass micropipette (tip outer diameter, 30–40 μm) filled with PSS-albumin solution, and the outside of the arteriole was securely tied to the pipette with 11-0 ophthalmic suture (Alcon, Fort Worth, TX). The other end of the vessel was cannulated with a second micropipette and also secured with a suture. After cannulation, the vessel and pipettes were transferred to the stage of an inverted microscope (model CKX41; Olympus) coupled to a video camera (Sony DXC-190; Labtek, Campbell, CA), video micrometer (Cardiovascular Research Institute, Texas A&M University System Health Science Center, College Station, TX) and a data-acquisition system (PowerLab; ADInstruments, Colorado Springs, CO) for continuous measurement and recording of the internal diameter throughout the experiment.[19] The micropipettes were connected to independent pressure reservoirs. Adjustment of the height of the reservoirs pressurized the vessel to 55 cm H_2O intraluminal pressure without flow. This level of pressure was used based on pressure ranges that have been documented in retinal arterioles in vivo[20] and in the isolated, perfused retinal microcirculation.[21] Preparations with side branches and leaks were excluded from further study.

Experimental Protocols

Cannulated arterioles were bathed in PSS-albumin at 36 to 37°C to allow development of basal tone. After vessels developed a stable basal tone (~30–40 minutes), the dose-dependent vasodilation response to neutralized L-lactate (0.01–10 mM) was constructed. After completing the control responses, the vasodilation elicited by L-lactate was reexamined after 30 minutes, to confirm the reproducibility of the response. Some vessels were also exposed to neutralized D-lactate (10 mM). The vessels were exposed to each concentration of agonists for 3 to 5 minutes until a stable diameter was established.

To elucidate the possible signaling mechanisms involved in the retinal arteriolar dilation response to lactate, the following series of experiments were performed. The role of lactate transport in the vasodilatory response to lactate was established in the presence of the monocarboxylate transporter inhibitor α-cyano-4-hydroxycinnamate (CHC, 10 μM).[22,23] The involvement of prostaglandins and NO in mediating the vascular response was assessed in the presence of known effective concentrations of the specific inhibitors indomethacin (10 μM)[24,25] and N^G-nitro-L-arginine methyl ester, (L-NAME, 10 μM),[19] respectively. The role of guanylyl cyclase/cGMP signaling was assessed by incubation with soluble guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ, 0.1 μM)[26] and the cGMP phosphodiesterase inhibitor zaprinast (1 μM).[27] To confirm the efficacy of ODQ, we examined the vasodilation response to guanylyl cyclase activator *S*-nitroso-*N*-acetylpenicillamine (SNAP; 0.1 μM to 0.1 mM).[28] The contribution of K_{Ca} and ATP-sensitive potassium (K_{ATP}) channels was assessed in the presence of known effective concentrations of the specific inhibitors iberiotoxin (0.1 μM)[25] and glibenclamide (5 μM),[19] respectively. In a separate series of experiments, we studied the effect of glibenclamide (5 μM), in combination with ODQ (0.1 μM), on SNAP-induced vasodilation. All drugs were administered extraluminally, and each antagonist was incubated for at least 30 minutes.

Chemicals

Drugs were obtained from Sigma-Aldrich (St. Louis, MO), except when stated otherwise. L-NAME and SNAP were dissolved in PSS and water, respectively. Neutralized lactate was prepared by dissolving lactic acid in water followed by adjustment of the pH to 7.4 with NaOH (10 N). Indomethacin, ODQ, CHC, and pinacidil were dissolved in ethanol, and glibenclamide was dissolved in dimethylsulfoxide as stock solutions (10 mM). Subsequent concentrations of these drugs were diluted in PSS. The final concentration of ethanol or dimethylsulfoxide in the vessel bath was 0.1%. Vehicle control studies indicated that this final concentration of solvent had no effect on the arteriolar function.

Data Analysis

At the end of each experiment, the vessel was relaxed in an EDTA (1 mM)-calcium-free PSS to obtain its maximum diameter at 55 cm H_2O intraluminal pressure. All diameter changes in response to agonists were normalized to this maximum vasodilation and expressed as a percentage of maximum dilation.[19] Data are reported as the mean ± SEM and n values represent the number of vessels studied. In each set of interventions, the vessels have their own control, with each vessel being from a different eye. The control vessels were pooled when comparing a series of experiments examining the effect of antagonists on vasodilation. Statistical comparisons of data were performed by Student's *t*-test or by analysis of variance followed by the Bonferroni multiple-range test, as appropriate. $P < 0.05$ was considered significant.

Results

Vasodilation of Retinal Arterioles to Lactate

In this study, all vessels ($n = 49$) developed a similar level of basal tone (constricted to 62% ± 2% of their maximum diameter) at a 36 to 37°C bath temperature with 55 cm H_2O intraluminal pressure. The average resting and maximum diameters of the vessels were 67 ± 2 and 104 ± 2 μm, respectively. Neutralized L-lactate (10 mM) produced a robust dilation of an isolated arteriole from the baseline diameter of 52 to 68 μm (Fig. 1A). The diameter gradually returned to the baseline level after the vessel bath was replaced with PSS (Fig. 1A). Further study showed that L-lactate produced concentration-dependent dilation of retinal arterioles that was reproducible and did not deteriorate after repeated application (Fig. 1B). In general, the dilation of arterioles to each concentration of L-lactate was developed within 20 to 30 seconds, and the highest concentration (10 mM) of L-lactate elicited nearly 70% of maximum dilation (Fig. 1B). The monocarboxylate transporter inhibitor CHC significantly attenuated the vasodilation ($P = 0.001$) in response to L-lactate (Fig. 1B). The ability of lactate to induce vasodilation was greater for the physiological L-lactate because neutralized D-lactate (10 mM) produced only 18% ± 2% dilation ($P = 0.006$; $n = 5$). Notably, we measured the pH of the L- and D-lactate solutions in the vessel bath and did not detect a significant change, suggesting that the observed effect of lactate on vascular tone was independent of extracellular pH.

Role of Endothelium-Derived Factors in Retinal Arteriolar Dilation Elicited by Lactate

The relative contribution of NO and cyclooxygenase-derived prostaglandins to lactate-induced vasodilation was examined and compared in the absence and presence of their respective inhibitors. The NO synthase inhibitor L-NAME almost completely inhibited the vasodilatory response (Fig. 2). In contrast, cyclooxygenase inhibitor indomethacin only reduced the vasodilation by ~15% at the highest concentration of lactate (Fig. 2). The resting basal tone was significantly increased by L-NAME (control: 60% ± 1% versus L-NAME: 53% ± 3%; $P = 0.04$) but not by indomethacin (control: 57% ± 2% versus

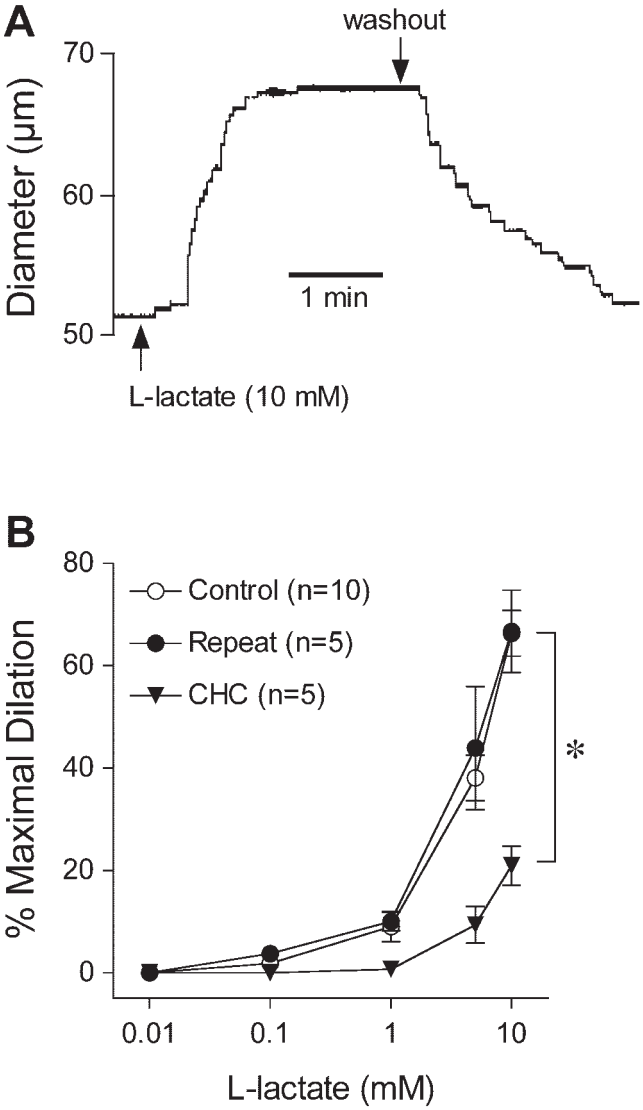

FIGURE 1. Vascular reactivity of isolated retinal arterioles to lactate. (**A**) Representative tracing shows L-lactate (10 mM)-induced dilation of retinal arterioles that was sustained for 3 minutes. The diameter returned to baseline level after washout. (**B**) Dose-dependent vasodilation to L-lactate was examined (control; resting diameter: 69 ± 6 μm; maximum diameter: 107 ± 5 μm) and then repeated after a 30-minute washout period in the absence (repeat; resting diameter: 71 ± 6 μm; maximum diameter: 113 ± 6 μm) or presence of the monocarboxylate transporter inhibitor CHC (10 μM; resting diameter: 64 ± 11 μm; maximum diameter: 102 ± 9 μm). $^*P < 0.05$ versus control.

indomethacin: 56% ± 2%; $P = 0.09$). In the presence of both indomethacin and L-NAME, the vasodilatory response to lactate was abolished (Fig. 2).

Role of Guanylyl Cyclase and cGMP Pathways in Retinal Arteriolar Dilation Response to Lactate

We next examined the contribution of the guanylyl cyclase and cGMP cascade to lactate-induced vasodilation. As shown in Figure 3, the soluble guanylyl cyclase inhibitor ODQ significantly reduced the vasodilation response to lactate ($P = 0.001$) in a manner similar to that of L-NAME. In contrast, the cGMP phosphodiesterase inhibitor zaprinast increased the dilation response to lactate ($P = 0.01$), especially at the higher concentrations (>1 mM). The resting basal tone was slightly increased by ODQ (control: 66 ± 1% versus ODQ: 59 ± 1%; $P = 0.004$) but was unaltered by zaprinast (control: 63% ± 2% versus zaprinast: 64% ± 2%; $P = 0.35$).

Role of K_{Ca} and K_{ATP} Channels in the Retinal Arteriolar Dilation Response to Lactate

To determine the relative contribution of K_{Ca} and K_{ATP} channels in lactate-mediated vasodilation, dose-dependent responses to lactate were examined in the absence and presence of their respective inhibitors iberiotoxin and glibenclamide. As shown in Figure 4, iberiotoxin did not alter the vasodilation response to lactate, whereas glibenclamide almost completely blocked the response. Glibenclamide did not alter resting basal tone (control: 67% ± 3% versus glibenclamide: 66% ± 2%; $P = 0.84$).

Role of Guanylyl Cyclase Activation in K_{ATP} Channel-Mediated Vasodilation

Because NO/cGMP pathway and K_{ATP} channels are involved in the lactate-induced vasodilation as just described, it is plausible that the opening of K_{ATP} channels is mediated by the activation of NO/cGMP signaling. To explore this possibility, we examined whether the dilation of retinal arterioles to an NO donor SNAP (i.e., activation of cGMP pathway) can be blocked by the K_{ATP} channel inhibitor glibenclamide. As shown in Figure 5, SNAP produced concentration-dependent dilation of retinal arterioles, and this dilation was significantly attenuated by ODQ ($P = 0.001$). In another group of vessels, glibenclamide inhibited SNAP-induced vasodilation in a similar manner similar to that of ODQ ($P = 0.001$). Combined administration of ODQ and glibenclamide did not further reduce SNAP-induced vasodilation. These results suggest that ODQ and glibenclamide inhibit the same vasodilatory pathway and that activation of the

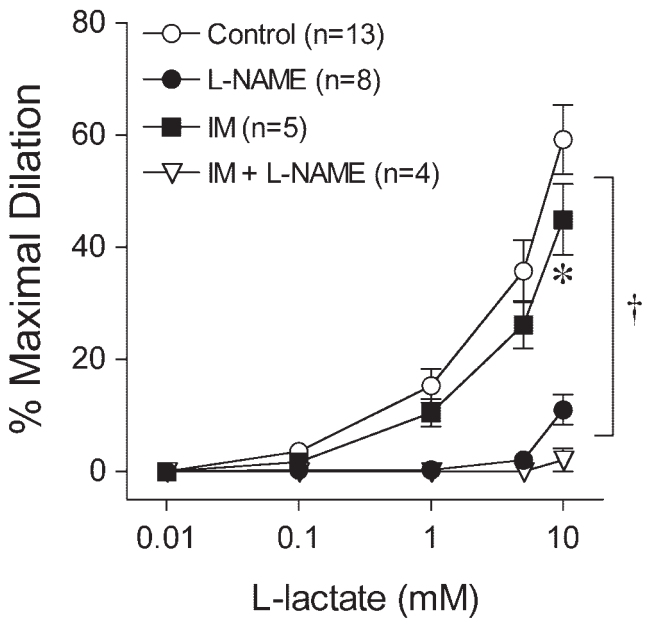

FIGURE 2. Role of endothelium-derived factors in the isolated retinal arteriolar dilation response to lactate. Dose-dependent vasodilation to L-lactate was examined before (control; resting diameter: 62 ± 3 μm; maximum diameter: 108 ± 2 μm) and after incubation with the NO synthase inhibitor L-NAME (10 μM; resting diameter: 55 ± 5 μm; maximum diameter: 102 ± 5 μm) and the cyclooxygenase inhibitor indomethacin (IM, 10 μM; resting diameter: 58 ± 5 μm; maximum diameter: 104 ± 7 μm) alone or in combination (resting diameter: 61 ± 5 μm; maximum diameter: 110 ± 3 μm). $^*P < 0.05$ versus control; †$P < 0.05$ versus control or indomethacin.

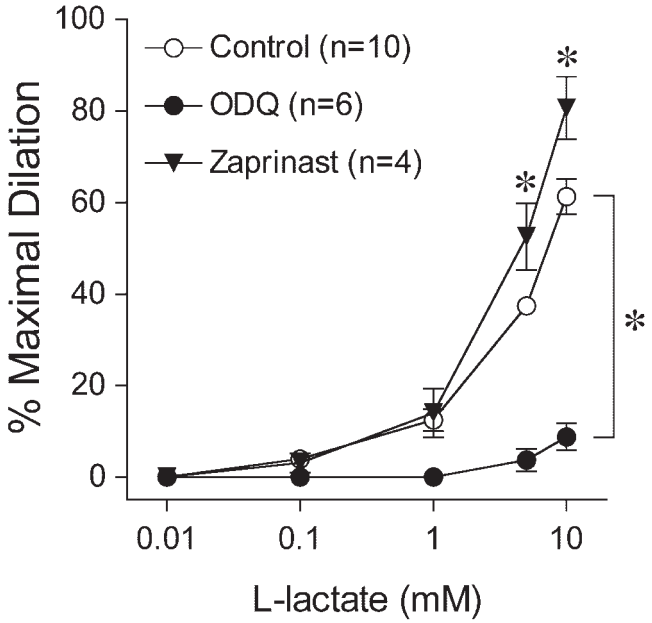

FIGURE 3. The role of guanylyl cyclase/cGMP signaling in the isolated retinal arteriolar dilation response to lactate. Dose-dependent vasodilation in response to L-lactate was examined before (control; resting diameter: 60 ± 3 μm; maximum diameter: 92 ± 5 μm) and after incubation with the soluble guanylyl cyclase inhibitor ODQ (0.1 μM; resting diameter: 56 ± 5 μm; maximum diameter: 95 ± 6 μm) or the cGMP phosphodiesterase inhibitor zaprinast (1 μM; resting diameter: 57 ± 4 μm; maximum diameter: 89 ± 7 μm). *$P < 0.05$ versus control.

NO/cGMP signaling leads to K_{ATP} channel-mediated dilation in retinal arterioles.

DISCUSSION

A change in retinal arteriolar diameter in response to vasoactive metabolites is essential in the regulation of retinal blood flow to match the metabolic demands of retinal tissue.[8] Lactate is a major product of retinal tissue metabolism that has been proposed to be an essential signal for modulating retinal microvascular tone. The putative regulatory role of lactate is based on the observations in animal and human studies showing that intravitreal[6] and intravenous[9,10] administration of L-lactate, as well as exercise-induced hyperlactatemia,[29] evokes dilation of retinal arteries and increases retinal blood flow, respectively, in vivo. Our present results provide the first direct evidence that L-lactate, independent of pH, causes dilation of isolated porcine retinal arterioles. We also demonstrate that the L-lactate-induced vasodilatory response is mediated by stimulation of NO synthase and subsequent activation of guanylyl cyclase, leading to the opening of K_{ATP} channels.

Although previous in vivo studies have shown that lactate causes dilation[6] and increases blood flow,[9,10] the unequivocal determination of the direct role of lactate in vascular regulation in vivo is difficult, because diameter changes and blood flow regulation in this situation are mingled with humoral, hemodynamic (i.e., myogenic and flow-induced responses), and local metabolic control mechanisms. Another caveat for interpreting the results of these in vivo studies is the inability to measure diameter changes in small retinal arterioles due to the limited resolution of the detection instrument.[6,10] It is also difficult to distinguish between the effect of lactate and pH on vascular tone. Because lactate with a pK_a of 3.86 is more than 99% dissociated under normal physiological conditions of pH 7.4,[30] it is possible that the accompanying acid pH in the retinal tissue contributes to the vasodilator phenomenon observed in vivo with lactate administration. In contrast, lactate may exert vasodilatory effects secondary to the interaction with retinal tissue. Indeed, acidosis has been shown to cause dilation of coronary[31] and cerebral[32] arterioles in vitro, and lactate can stimulate release of vasodilator adenosine from nonvascular tissues.[33] Therefore, in the present study, we avoided these potential confounding influences by characterizing the vasomotor response of small retinal arterioles to neutralized L-lactate (pH 7.4) under a defined environment using an isolated vessel preparation. Neutralized L-lactate caused significant dilation of isolated retinal arterioles at concentrations of 1 to 10 mM. This range of lactate concentrations is within the normal physiological retinal tissue level of approximately 7 mM[9] and may reflect pathophysiological levels during short-term hypoxia, which has been shown to increase retinal tissue production of lactate nearly 1.5-fold.[1] Although 10 mM D-lactate also caused dilation, the response was significantly less than that to L-lactate, the predominant physiological form of lactate in mammals.[34] Because the retina produces more lactate aerobically than any other tissue,[35] our results support the notion that L-lactate per se contributes significantly to the regulation of retinal microvascular tone in normal physiological conditions and possibly during acute hypoxia.

Signaling mechanisms that have been proposed to mediate the dilation to neutralized L-lactate in various tissue preparations include the activation of smooth muscle guanylyl cyclase[13,14,16] and potassium channels[18] without the contribution of endothelium-derived vasodilators. However, the putative mechanisms mediating L-lactate-induced dilation of retinal arterioles have not been determined. In the present study, we examined the possible role of endothelium-dependent vasodilators such as NO and prostaglandins in the lactate-induced vasodilation. The NO signaling pathway is worth investigating because endothelium-derived NO is a physiological activator of guanylyl cyclase. Furthermore, L-NAME has been shown to reduce the increased retinal blood flow in response

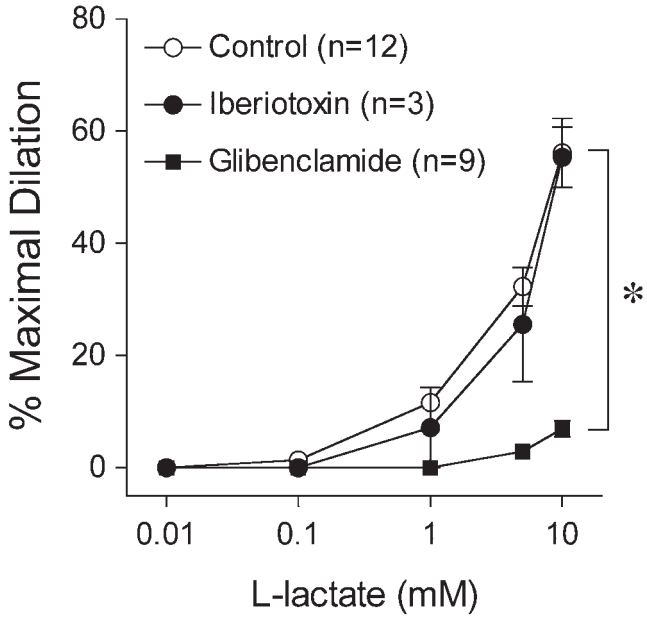

FIGURE 4. The role of K_{Ca} and K_{ATP} channels in isolated retinal arteriolar dilation response to lactate. Dose-dependent vasodilation in response to L-lactate was examined before (control; resting diameter: 75 ± 5 μm; maximum diameter: 111 ± 5 μm) and after incubation with the K_{Ca} channel inhibitor iberiotoxin (0.1 μM; resting diameter: 71 ± 1 μm; maximum diameter: 105 ± 8 μm) or the K_{ATP} channel inhibitor glibenclamide (5 μM; resting diameter: 76 ± 6 μm; maximum diameter: 114 ± 6 μm). *$P < 0.05$ versus control.

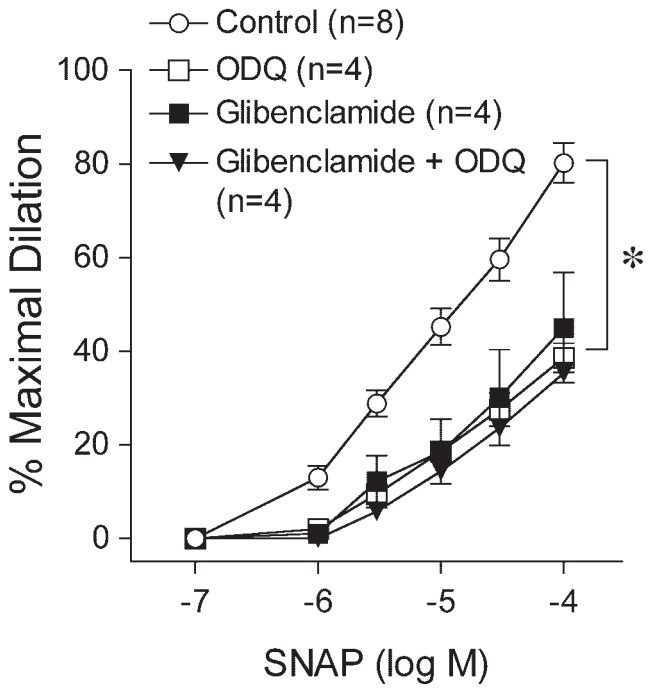

FIGURE 5. The effects of guanylyl cyclase and K_{ATP} channel blockade on the isolated retinal arteriolar dilation response to SNAP. Dose-dependent vasodilation to the NO donor SNAP was examined before (control; resting diameter: 76 ± 6 μm; maximum diameter: 119 ± 6 μm) and after incubation with ODQ (0.1 μM; resting diameter: 68 ± 9 μm; maximum diameter: 115 ± 6 μm) and glibenclamide (5 μM; resting diameter: 85 ± 4 μm; maximum diameter: 123 ± 11 μm) alone or in combination (resting diameter: 68 ± 10 μm; maximum diameter: 115 ± 6 μm). $^*P < 0.05$ versus control.

to intravenous administration of lactate in rats.[9] However, because it is difficult to exclude the potential changes in plasma pH and also to distinguish between neuronal- and vascular-mediated NO action in in vivo settings, the isolated vessel preparation provides the most appropriate approach for unambiguous identification of this signaling pathway. In a finding different from those in other organ systems,[13,14,16] we found that blockade of NO synthase with L-NAME almost completely inhibited the lactate-induced vasodilation, suggesting that NO contributes in large part to the response. It appears that prostaglandins are partially involved in the retinal arteriolar dilation response to the highest concentration of L-lactate (10 mM) because blockade of cyclooxygenase only reduced the response by approximately 15%. In the presence of both indomethacin and L-NAME, the lactate-induced vasodilation was completely inhibited. These findings appear to be unique to the retinal arterioles, because the lactate-induced dilation in other vascular beds has been shown to be independent of NO or prostaglandins.[13,14,16] The discrepancy between the previous findings and our observations is uncertain, but could reflect differences in species or vascular beds. The lack of an effect by indomethacin in an earlier in vivo study in the retinal circulation may be related to the intravitreal administration of a nonphysiological high concentration of lactate (i.e., 500 mM).[6] Nonetheless, our data provide the first direct evidence that both endothelium-derived NO and prostaglandins contribute to the lactate-induced dilation of small retinal arterioles, with NO signaling playing a predominant role.

In the present study, the selective soluble guanylyl cyclase inhibitor ODQ nearly abolished the retinal arteriolar dilation response to lactate. This inhibitory effect appeared to be specific for guanylyl cyclase because ODQ inhibited the vasodilation to NO donor SNAP (Fig. 5) but did not affect the vasodilation response to the direct K_{ATP} channel opener pinacidil ($n = 4$; data not shown). Further support for guanylyl cyclase activation was provided by the ability of the cGMP phosphodiesterase inhibitor zaprinast to enhance the vasodilation response to lactate. Zaprinast inhibits the degradation of cGMP in the vascular smooth muscle by inhibiting cGMP phosphodiesterase activity and thus increases the cellular level of cGMP, which has been shown to augment vasorelaxation to guanylyl cyclase activation in other blood vessels.[36,37] In the pilot studies, a high concentration of zaprinast (10 μM) produced 10% to 15% vasodilation ($n = 4$). To avoid the confounding effect of reducing vascular tone on lactate-induced vasodilation, we chose a lower concentration of zaprinast (1 μM) for the present study. Nevertheless, zaprinast, at a concentration that did not alter basal tone, was sufficient to enhance the vasodilation response to lactate. Taken together, our findings indicate that the activation of soluble guanylyl cyclase and subsequent increase in cGMP contributes to the retinal arteriolar dilation response to lactate. However, the precise mechanism contributing to activation of the NO/cGMP pathway by L-lactate remains unclear. A recent in vivo study in rats[9] suggests that retinal blood flow can be increased by transferring additional electrons and protons from lactate to NAD^+ (nicotinamide adenine dinucleotide). It seems that alteration of cytosolic redox state (i.e., increased NADH [reduced nicotinamide adenine dinucleotide dehydrogenase]/NAD^+ ratio linking to metabolic activation), can contribute to vasodilation associated with lactate production.[9] The increased retinal blood flow after visual stimulation is sensitive to NO synthase inhibition,[9] suggesting the potential link between redox signaling and NO synthase activation. Future studies are needed to determine whether this redox-signaling cascade is involved in the dilation of retinal arterioles in response to L-lactate.

Our results are also consistent with the idea that potassium channel activation, which can alter vascular tone by changing membrane potential, contributes to the lactate response in retinal microvessels. Specifically, our data show that retinal arteriolar response to lactate was almost completely inhibited by blockade of K_{ATP} channels. Of interest, these results are inconsistent with the previous observation that K_{Ca} channels mediate the dilation of porcine coronary arteries to lactate.[18] This discrepancy may be related to tissue specificity, because glibenclamide did not affect the dilation response of porcine coronary arterioles (~70 μm in diameter) to lactate (0.01–10 mM; $n = 3$, Hein TW, Kuo L, unpublished observations, 2005). Because the effect of K_{ATP} channel blockade was comparable to that produced by L-NAME and ODQ, it is possible that lactate induces a signaling pathway linking NO/cGMP to K_{ATP} channels. More specifically, we assumed that K_{ATP} channel activation is downstream of NO/cGMP signaling. If this were the case, we would expect to see the blockage of retinal arteriolar dilation to NO donors (via cGMP signaling) by glibenclamide. Indeed, similar to the effect of ODQ, glibenclamide inhibited the retinal arteriolar dilation response to the NO donors SNAP (Fig. 5) and morpholinosydnonimine ($n = 4$; data not shown). Because subsequent administration of ODQ to glibenclamide-treated vessels did not further reduce the response, it appears that these two signaling pathways (i.e., NO/cGMP and the K_{ATP} channel) are arranged in series in porcine retinal arterioles. This conclusion is supported by findings in certain vascular beds that guanylyl cyclase activation by NO donors can activate glibenclamide-sensitive K_{ATP} channels for hyperpolarization and vasodilation.[38,39] Patch-clamp studies also have reported that cGMP can activate K_{ATP} channels in cultured vascular smooth muscle cells isolated from the rat aorta.[40]

The cellular uptake of lactate has been shown to occur by facilitated transport via monocarboxylate transporters[41] or by passive diffusion.[42] The monocarboxylate transporters have been identified in plasma membranes of vascular endothe-

lial[43,44] and smooth muscle cells.[45,46] Of note, the monocarboxylate transporters were also localized on Müller cell foot processes surrounding retinal vessels,[44] which supports the idea that the production and release of lactate by these glial cells could influence vascular tone. Our results suggest that the monocarboxylate transporters play a major role in lactate-induced vasodilation because the cognate inhibitor CHC specifically reduced the response by approximately 80%. It is likely that the residual dilation resulted from L-lactate diffusion into the vascular cells.

A limitation of our study is the inability to characterize the vasomotor response of arterioles less than 30 μm in diameter, because of the technical difficulty in vessel preparation. Nevertheless, the range of vessel size from 43 to 95 μm with basal tone was investigated in the present study. Eight of the vessels were less than 50 μm in diameter, and all dilated in response to L-lactate. Small arterioles of this size can contribute significantly to the resistance and flow regulation in the retinal circulation, because a marked pressure drop (i.e., 50%) across these vessels was observed in the feline retina.[20] It should also be noted that the general application of the present findings in the retina should be interpreted with caution because the production and metabolism of lactate can vary depending on the species. For example, nearly 90% to 99% of the glucose used aerobically is converted to lactate in the rat retina[47] and human retinal Müller cells,[2] whereas 40% to 60% occurs in the rabbit[48] and porcine[49] retina.

In summary, the present study provides the first direct evidence that L-lactate causes dilation of retinal arterioles. The uptake of lactate by vascular cells via monocarboxylate transporters leads to stimulation of NO synthase and subsequent activation of guanylyl cyclase. The guanylyl cyclase and cGMP signaling result in K_{ATP} channel activation for vasodilation. It is worth noting that retinal tissue production of lactate is increased during acute retinal hypoxia,[50] and the increased retinal blood flow by hypoxia[51] or metabolic activation[9] is sensitive to NO synthase inhibition. Our current findings may provide mechanistic insight into the signaling pathways contributing to the vasodilation and augmented blood flow associated with hypoxia, as well as to the metabolic regulation of retinal blood flow under normal physiological conditions.

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