Microinjection of L-Lactate in the Preretinal Vitreous Induces Segmental Vasodilation in the Inner Retina of Miniature Pigs

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Purpose. The authors investigated the hypothesis that the retinal vasomotor effect of acute hypoxia is mediated by lactate.

Methods. Retinal vasomotor arteriolar response was measured in the intact eyes of miniature pigs after systemic administration and after local preretinal juxta-arteriolar microinjection of lactate.

Results. Injection of L-lactate (physiologically produced lactate) into the systemic circulation decreased the arterial blood pH but did not dilate the retinal arterioles. By contrast, microinjections of L-lactate (0.5 mol/l, pH 2) into the juxta-arteriolar vitreous induced a reversible segmental vasodilation of 32 ± 4% (standard deviation). This vasodilation did not depend on periarteriolar pH lowering because microinjections of a 0.5 mol/l L-lactate at neutral pH also dilated segmentally the retinal arterioles (37 ± 5.5%). The effect of lactate was stereospecific because microinjections of the isomer D-lactate (0.5 mol/l, pH 2) did not affect the arteriolar caliber (P = 0.63). Perfusion of the eye with the cyclo-oxygenase inhibitor indomethacin, through cannulization of the sublingual artery, caused a generalized reversible arteriolar vasconstriction of 51 ± 9.8% but did not inhibit the segmental vasodilator effect of locally microinjected L-lactate.

Conclusions. It is known that acute hypoxia in the isolated retina causes an increase in lactate production. In the intact eye, there is a retinal vasodilation, which is not inhibited by indomethacin. Hence, it was concluded that retinal, but not blood, lactate is a possible mediator of the acute hypoxia-induced vasodilation. Invest Ophthalmol Vis Sci 1993;34:1744-1752.

Acute hypercapnia and hypoxia induce important dilation of retinal arterioles in parallel with interstitial acidification in the inner retina.1-5 However, only the hypercapnia-induced vasodilation is suppressed by the cyclo-oxygenase inhibitor indomethacin,4,5 suggesting that the effect of hypercapnia is probably mediated by the release of prostaglandins. Indeed, juxta-arteriolar microinjection of prostaglandin E1 causes segmental dilation of retinal arterioles.4 In this article, we explore the hypothesis that the effect of acute hypoxia is not mediated by prostaglandins but by lactate. The formulation of this hypothesis is based on the biochemical evidence showing that the mammalian retina produces large quantities of lactate6 and that in the isolated superfused retina hypoxia increases the production of lactate.7 This article focuses on a central aspect of this hypothesis, namely, that lactate exerts a clear vasodilating effect on the retinal arterioles that might...
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depend on inner retinal pH changes and is not suppressed by indomethacin.

MATERIALS AND METHODS

Experiments were performed on the intact eye of miniature pigs weighing 9–11 kg (Göttingen breed, Arare, Geneva, Switzerland). The retina of miniature pigs is an experimental model close to the human retina in both neuroanatomic and vascular aspects.6–10 The surgical preparation of the animals has been extensively described in previous reports.11–13 In brief, the animals were premedicated by intramuscular injection of 40 mg of azaperon (Stresnil, Cilag Chemie, AG) and anesthetized with intravenous injection of approximately 30 mg of metomidate chloride and 1 mg of atropine. They were then paralyzed (tubocurarine, 1 mg/10 kg), intubated, and artificially ventilated with a mixture of O₂ and N₂O. The gas mixture, stroke volume, and stroke frequency were adjusted, according to physiologic values of arterial partial oxygen and carbon dioxide pressures and pH, which were monitored throughout the experiment with a blood gas analyzer (AVL [Roswell, GA] automatic blood gas system 940). Systolic and diastolic blood pressure were continuously checked using the femoral artery with a transducer (Minograph, Siemens-Elema, Sweden). The rectal temperature was maintained between 36–37°C. All experiments reported in this article were done at steady state (partial oxygen pressure, 12–13.5 kPa; partial carbon dioxide pressure, 4–6 kPa; pH 7.37–7.45; systolic blood pressure, 110–120 mmHg).

The animals were treated according to the ARVO Resolution on the Use of Animals in Research.

Ocular Surgery

The eyelids were removed, and the sclera around the limbus was prepared. A metal ring was sutured to the limbus and attached to a goose-neck manipulator5 in a fixed position to immobilize the eye. The pupil was dilated with phenylephrine 5%, tropicamide 0.5%, and atropine 1% eye drops. The cornea was protected with diluted sodium hyaluronate and fitted with a vitreoretinal surgery contact lens on which a fiberoptic cable was placed to illuminate the fundus. Fundus observation was performed with an operating Zeiss microscope.

Perfusion of the Eye Through Cannulization of the Sublingual Artery

In pigs, the sublingual artery, which runs near the hyoid bone, is a branch of the external carotid artery.5 The ophthalmic artery derives from the external carotid artery immediately after the entrance of the latter into the skull. The distance between the issue of the sublingual artery and the emergence of the ophthalmic artery is approximately 3 cm. Consequently, it is possible to perfuse the eye with different solutions by introducing a catheter in the sublingual artery and by obtaining, through an occluding ligature, a retrograde flow toward the external carotid and the ophthalmic artery. This surgical preparation was done for the perfusion of the eye with L-lactate and indomethacin.

pH Microelectrodes

In some experiments, the juxta-arteriolar pH was measured with pH-sensitive microelectrodes. When pH measurements were combined with pressure microinjections, the pH-sensitive microelectrode was attached to a second micromanipulator and was introduced into the eye through a second sclerotomy. More information concerning the pH-sensitive microelectrodes can be found in the literature.5,11

Pressure Microinjections

Micropipettes (tip diameter, 3–4 μm) were pulled from borosilicate capillaries. A micropipette can contain up to 10 μl of solution. The pressure microinjection method developed earlier was used.14 The micropipettes were tested before their introduction in the vitreous cavity; in general, an air pressure of approximately 50 psi applied to the solution in the micropipette for less than 1 sec was sufficient for the injection of approximately 0.2 μl of solution. The micropipette, which was firmly attached to an electronic micromanipulator,15 was introduced in the vitreous cavity through a pars plana sclerotony and placed at a distance of approximately 50–100 μm from a retinal arteriole, usually in the superotemporal retinal sector. In all experiments, one single juxta-arteriolar microinjection of a solution of lactate was performed. Because no vitreous loss occurred from the surgical wound, there was no decrease in the intraocular pressure during the experimental procedure.

Photographic Setup

When the micropipette was placed near a retinal arteriole, the hard contact lens was replaced by a soft contact lens, the fiberoptic cable was removed, and observation of the retinal area where the micropipette was placed was done with a Zeiss fundus camera using red-free light. Care was taken to have the arteriole close to which the micropipette was placed in focus. In experiments in which substances were injected into the sys-
In the systemic circulation, the superotemporal retinal arteriole was photographed. Experiments were documented with a series of red-free photographs starting 2 min before the administration of the tested solutions. Fundus photography (once every minute) was stopped after fundus observation showed recovery of the arteriolar diameter. Alternatively, in experiments in which arteriolar caliber changes were not observed, fundus photography was continued until 20 min after the microinjection. Some experiments were documented by fundus fluorescein angiography; 0.25 ml of a 20% aqueous solution was injected every 4 min into the femoral vein, and the same photographic sequence was followed. All microinjections were performed after the animal was in the steady state for at least 15 min. For all experimental procedures, no further focus adjustment was done with the fundus camera during the photographic sequence.

**Chemicals**

L-lactate was purchased from Fluka Chemie (Buchs, Switzerland) and indomethacin and D-lactate, from Sigma Chemicals (St. Louis, MO). Indomethacin was dissolved in methanol and then added to Ringer’s solution containing 124 mmol/l NaCl, 5 mmol/l KCl, 2 mmol/l CaCl₂, 1.25 mmol/l KH₂PO₄, 20 mmol/l NaHCO₃, and 10 mmol/l of HEPES buffer (N-[2-Hydroxyethyl]-piperazine-N'-2-ethane-sulfonic acid). The final solution contained 0.1% methanol.

**Arteriolar Caliber Measurements**

Arteriolar caliber measurements were done with a digital display slide caliper on projected (file projector, Slidex, Tokyo, Japan; ×10 magnification) fundus transparencies. The arteriolar diameter was measured separately by two observers on the same photograph, and the mean value of the two measurements was calculated. Caliber measurements were performed at the site of the microinjection. In experiments in which L-lactate or indomethacin was injected into the systemic circulation caliber, measurements were made on the superotemporal retinal artery approximately one disc diameter away from the optic nerve head. The mean intraobserver variability of the caliber measurements was 7%. The results are expressed as the mean percent retinal arteriolar caliber change ± the standard deviation. Statistical analysis was performed with the paired Student’s t test.

**RESULTS**

**Systemic Injection of L-Lactate**

In three miniature pigs, L-lactate (0.1 mol/l, pH 1.5) was slowly injected into the femoral vein. When the arterial blood pH decreased by 0.1 units, the injection was stopped. This pH decrease was equivalent to that occurring during systemic hypoxia.³ The increase in blood lactate and the concomitant systemic acidosis had no effect on retinal arteriolar diameter ($P = 0.56$). In one experiment, a pH-sensitive microelectrode was introduced into the eye and positioned in the preretinal vitreous close to the superotemporal arteriole. Despite the systemic acidosis caused by the lactate injection, the juxta-arteriolar pH remained unchanged. A pulse of hypercapnia induced the expected drop in pH,³ confirming that the electrode responded to pH changes.

A different experimental protocol was used in another three animals. The eye was continuously perfused, through cannulization of the sublingual artery (1.6 ml/min), with L-lactate (0.1 mol/l, pH 2) for a period of 20 min. This procedure clearly induced a high lactate concentration in the blood flowing in the retinal circulation. However, even under this experimental condition, we did not observe vasomotor changes of the retinal arterioles.

**FIGURE 1.** Percentage increase in arteriolar diameter as a function of time after a juxta-arteriolar preretinal microinjection of L-lactate (0.5 mol/l, pH 2). Each symbol represents the responses in a different animal. The curve connects the mean calculated values.
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In these experiments, we performed preretinal juxta-arteriolar microinjections of L-lactate. Microinjections of 0.05 and 0.1 mol/l of L-lactate at pH 2 induced variable dilation of retinal arterioles. By contrast, microinjections of a 0.5 mol/l solution of L-lactate (pH 2), carried out on seven miniature pigs, induced reproducible segmental dilation of retinal arterioles (maximal effect, 32 ± 4%). This vasodilation was detectable shortly after the microinjection, and the maximal effect occurred 3–6 min after the microinjection. The segmental arteriolar dilation was reversible thereafter (Fig. 1). A typical experiment is documented in Figure 2. Microinjection of the solvent, which was used for the preparation of the solution of lactic acid, did not produce any detectable effect on the retinal arteriolar caliber.

The Role of Periarteriolar pH Modification on the Vasomotor Effect of Lactate

To evaluate the acidification of the prearteriolar vitreous, we measured the local pH using pH-sensitive mi-
microelectrodes during and after microinjection of L-lactate. In two animals, microinjection of a 0.5 mol/l solution of L-lactate at pH 6.9 induced a decrease of 0.4 units of pH. We therefore made juxta-arteriolar pressure microinjections of a 0.5 mol/l solution of L-lactate at pH 7.4. These microinjections induced again a segmental and reversible arteriolar dilation whose amplitude (37 ± 5.5%, n = 4) was similar to that observed after the microinjection of the acid solution. Figure 3 illustrates a typical example of this effect.

We further explored the role of pH in the lactate-induced vasodilation by performing microinjections of D-lactate (0.5 mol/l aqueous solution at pH 2). This experiment was repeated seven times on different animals. Preretinal microinjections (Fig. 4) of this unnatural isomer caused a decrease of juxta-arteriolar pH but did not modify the arteriolar caliber (P = 0.63).

The Effect of Indomethacin on the Vasomotor Effect of Lactate

Retinal Vasomotor Effect of Indomethacin. In five animals, the eye was perfused with indomethacin (7
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**FIGURE 4.** Microinjection of D-lactate (0.5 mol/l, pH 2). (A) Control photograph. The white arrow shows the micropipette crossing over the retinal vein. The black arrow shows the arteriolar site toward which the tip of the micropipette is directed. (B) Photograph taken during the microinjection showing the change of the refractive index. (C) Four minutes after the juxta-arteriolar microinjection, the arteriolar diameter has not changed. The micropipette has been removed from the eye.

$\times 10^{-4}$ mol/l, pH 7.4) through cannulization of the sublingual artery using a constant flow of 1.6 ml/min. Within the first 5 min of perfusion, a generalized arteriolar constriction (mean maximal effect, $51 \pm 9.8\%$) was observed (Fig. 5). The effect of indomethacin was completely reversed after 9–14 min of continuous perfusion (Figs. 5, 6), similar to hypocapnia-induced vasoconstriction. A representative experiment is shown in Figure 6. Perfusion of the eyes of three animals with the solvent used for the solution of indomethacin (Ringer's pH 7.4 solution containing 0.1% of methanol) did not affect the retinal arteriolar caliber.

**Perfusion of the Eye With Indomethacin and Juxta-Arteriolar Microinjection of L-Lactate.** Perfusion of the eye with indomethacin ($7 \times 10^{-4}$ mol/l) was initiated simultaneously with a pressure microinjection of L-lactate (0.5 mol/l, pH 2). After 2–3 min of perfusion at arteriolar sites away from the location of the microinjection, a generalized vasoconstriction was observed. By contrast, at the site of the microinjection, there was
The experimental evidence we presented supports the hypothesis that, in the acute hypoxia-induced dilation of retinal arterioles, a key step is the increase of L-lactate production by retinal cells. First, the microinjection of L-lactate in the vitreoretinal space induced a segmental vasodilation comparable to that induced by profound hypoxia, which is known to cause a rise in lactate production by the retina. When lactate was injected into the vitreoretinal space, it caused acidification of the preretinal vitreous. Two lines of experimental evidence presented in this article strongly suggest that the vasodilatory effect of lactate is not mediated by pH changes. First, microinjections of both acid and neutral solutions of L-lactate induced segmental vasodilation of similar amplitude, and second, microinjection of an acid solution of D-lactate caused acidification but no vasodilation. In general, lactate crosses cytoplasmic membranes by simple diffusion of its undissociated lipophilic free-acid form. Because its pK is 3.86, lactate is 99% dissociated at physiologic pH. Consequently, diffusion of lactate probably does not play an important role in the observed vasodilation because both a neutral pH solution of L-lactate, containing less than 1% of undissociated form, and a pH 2 solution, representing more than 99% in the undissociated form, induced similar vasomotor changes. In addition, a transporter of lactate has been described. If lactate injected into the vitreoretinal space exerted its effect directly on the smooth muscle.
of the arteriolar wall, it would have to cross the interfering glial and other cytoplasmic membranes using both mechanisms because the dilatory effect was observed at acidic and neutral pH. The absence of an effect of D-lactate is a result of either a perfect stereospecificity of the transporter or of no enzymatic recognition of D-lactate in the biochemical pathways.

The Effect of L-Lactate Is Not Mediated by Prostaglandins

The experimental data presented here are not sufficient to decide whether lactate exerts its effect directly on the smooth muscle or indirectly, through the release of a mediator. In this article, we explored only the role of prostaglandins. The experimental evidence showing that perfusion of the eye with the prostaglandin inhibitor, indomethacin, induced vasoconstriction, except at the site where lactate was injected, excludes prostaglandins as mediators of the effect of lactate and therefore of hypoxia, confirming previous results.4,5 Apparently, prostaglandins are mediators of another pathway that contributes in the maintenance of vasomotor tone in the retina. It has previously been shown that the level of the partial pressure of carbon...
dioxygen in the arterial blood affects this pathway. The vasoconstriction induced by indomethacin, like the vasoconstriction induced by profound hypocapnia, was not maintained despite a continuous perfusion of the eye with the drug or, in the case of hypocapnia, the low partial pressure of carbon dioxide. It is expected that vasoconstriction leads to a strong reduction of blood flow and, in turn, to tissue hypoxia. In the case of hypocapnia, the tissue hypoxia as measured with $O_2$-sensitive microelectrodes can be severe. We propose that acute hypoxia triggers a prostaglandin-independent pathway, leading to the increase of lactate, and, in turn, to the relaxation of retinal arterioles. This suggests the interesting possibility that the regulation of the arteriolar tone and, therefore, of blood flow in the inner retina is controlled by the interplay of at least two, apparently independent, metabolic pathways operating in the cells of the inner retina. Currently, there are no data excluding the existence of a biochemical link between these pathways.

**Key Words**

hypoxia, lactate, prostaglandins, retinal metabolism, retinal vasomotoricity

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**References**