Effects of Sodium Lactate on Isolated Rabbit Corneas

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Corneal stromal lactate accumulation may result from epithelial hypoxia and contact lens wear, but the possible corneal toxicity of lactate has not been reported. Isolated superfused whole rabbit corneas were examined for thickness changes during exposure to neutral sodium lactate (NaL) or excess sodium chloride (NaCl) in Krebs-bicarbonate Ringer’s solution for a 3-hr period. Placed in the tears side bath, 5 mM NaL significantly thinned corneas (swelling rates of 1 ± 1 μm/hr in Ringer’s controls vs -11 ± 1 μm/hr in lactate-treated corneas; mean ± SD). Excesses of 5 mM NaCl had essentially identical effects (0 ± 1 μm/hr in controls vs -13 ± 3 μm/hr in experimentals). When placed on the aqueous side of normal-thickness corneas, neither 20 mM NaL nor 20 mM excess NaCl affected corneal thickness, but both solutions stimulated endothelium-mediated deswelling in preswollen de-epithelialized corneas. When “loaded” into the stroma of deepithelialized corneas, Ringer containing 20 mM lactate caused more swelling than Ringer’s alone (491 ± 18 μm in controls vs 558 ± 20 μm in loaded corneas; mean ± SEM). A similar swelling occurred when 20 mM excess NaCl was loaded into the stroma (483 ± 15 vs 565 ± 20 μm in controls and loaded corneas, respectively), due to fluid uptake into the hypertonic stroma across the endothelium from the aqueous side (Ringer’s) bath. Corneas both loaded and superfused with either NaL or excess NaCl swelled and subsequently deswelled similar to controls swollen and superfused in Ringer’s. These experiments demonstrate that like NaCl, NaL up to 20 mM appears to be nontoxic to the cornea, but can osmotically affect corneal thickness, which can acutely account for hypoxic corneal edema.

Materials and Methods

Male and female New Zealand White rabbits (2–3 kg) were sacrificed with a pentobarbital overdose via the marginal ear vein, and the corneas were mounted for specular microscopy in isolated superfused rabbit corneas. To determine the acute effects of lactate from the tears, aqueous, and stroma, these studies examined thickness changes by means of specular microscopy in isolated superfused rabbit corneas.

Materials and Methods

Male and female New Zealand White rabbits (2–3 kg) were sacrificed with a pentobarbital overdose via the marginal ear vein, and the corneas were mounted for specular microscopy as described by Dikstein and Maurice.15 Except where noted (see Preswollen Corneas and Lactate-“Loaded” Corneas below), corneas were mounted with epithelium and endothelium intact. This investigation adhered to the ARVO Resolution on the Use of Animals in Research. On the aqueous side (chamber volume = 0.25 ml), the corneas were maintained with a pressure of 20 cm H2O and a flow rate of 0.5–1.5 ml/hr. The tears side bath (chamber volume = 0.5 ml) was changed every 30 min. The bathing solutions were bubbled with 95% O2 and 5% CO2 to maintain a pH of 7.3–7.4 on the aqueous side, and 7.3–7.8 on the tears side. The bathing solution used was Krebs–bicarbonate Ringer’s (118 mM NaCl, 4.7 mM KCl, 1.9 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, and 27.8 mM glucose) with 0.5 mM adenosine and 0.3 mM reduced glutathione. Osmometry

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was performed on each bathing solution with a Precision Systems Osmette A Osmometer, and all solutions had an osmolality of 300 ± 5 mOsm/kg before sodium lactate (NaL) or NaCl addition. Addition of 5 mM NaCl or NaL increased the osmolality to 310 ± 2 mOsm/Kg; 20 mM NaCl or NaL increased the osmolality to 340 ± 2 mOsm/Kg. All bathing solutions were made weekly and kept refrigerated, and all added agents (NaL, excess NaCl, adenosine, or glutathione) were added on the day of use. Reagents were obtained from Fisher Scientific (St. Louis, MO) except for adenosine, glutathione, and lactic acid, which were obtained from Sigma (St. Louis, MO). Lactic acid was converted to a 2 M NaL solution by equimolar titration with NaOH, and was kept frozen for no longer than 5 weeks.

Normal-Thickness Corneas

After a 1.5-hr equilibration period in the Ringer’s solution, corneal thickness was monitored for a 3-hr experimental period. At the beginning of the 3-hr period (zero time), control and experimental corneas typically differed in thickness by less than 10 μm. If the difference exceeded 30 μm, the pair was rejected, based on apparent differences in initial corneal swelling pressure. At zero time, Ringer’s containing NaL or excess NaCl was placed on the experimental corneas in either the tears side or aqueous side bath, and the paired control cornea was bathed in normal Ringer’s. The thickness of each cornea was measured in duplicate at zero time and every 30 min (a total of 14 observations for each cornea). For each group of five to eight corneas, least squares linear regression analysis was conducted to determine the group swelling rate and its standard deviation. Group swelling rates were compared by analysis of covariance at the P < 0.05 level of significance as described by Hull et al.18

Preswollen Corneas

Corneas were deepithelialized with a Gill corneal knife and swollen with Ringer’s on the denuded surface. After a thickness of approximately 560–580 μm was reached (approximately 1 hr), the solution was removed and blotted away from the stromal surface, and was replaced with silicone oil (Dow-Corning 360 Medical Fluid, 20 centistokes) to prevent further fluid exchange. After a 30–45-min equilibration under the oil, thickness was monitored for a 3-hr experimental period. This enabled an examination of deturgescence in the presence of NaL or excess NaCl in the aqueous bath.

Lactate-“Loaded” Corneas

Corneas were deepithelialized (See Preswollen Corneas, above) and exposed to Ringer’s (controls) or Ringer’s containing 20 mM NaL or excess NaCL (experiments) to “load” the stroma with either Ringer’s or Ringer’s made hypertonic with NaL or NaCL. This enabled an examination of their effects on thickness and endothelium-mediated deturgescence. In one type of loading experiment, the aqueous bath contained Ringer’s without NaL or excess NaCl. In a second protocol, the aqueous bath contained NaL or NaCl Ringer’s identical to that loaded into the stroma. Thus, the second loading experiments were designed to minimize lactate gradients across the endothelium during deturgescence.

Lactate Assay

The corneas loaded with lactate by the first protocol were assayed for their concentration as described
Fig. 3. NaL (20 mM) in the aqueous-bath-stimulated endothelium-mediated deturgescence in preswollen deepithelialized corneas (n = 6). *P < 0.01.

by Klyce, immediately after loading and measurement of thickness, or after 1.5 hr of deswelling. Lactate was assayed by the method of Bergmeyer using the Sigma lactate assay kit (Sigma catalog no. 826 UV), which utilizes beef heart lactate dehydrogenase to convert lactate to pyruvate. Pyruvate is trapped as a hydrazone to enable quantitative conversion of NADH to NAD, which is measured spectrophotometrically at 340 nm.

Results

Effects of Lactate in the Aqueous Bath

When placed on the aqueous side, Ringer's containing 20 mM NaL had no significant effect on corneal thickness or swelling rates compared to normal Ringer's (Fig. 1), and a similar finding was observed with 20 mM excess NaCl (Fig. 2). In addition, 20 mM NaL or excess NaCl apparently were not toxic to endothelium-mediated deturgescence in preswollen deepithelialized corneas (Figs. 3, 4); both agents stimulated deturgescence.

Fig. 4. Excess NaCl (20 mM) in the aqueous-bath-stimulated endothelium-mediated deturgescence in preswollen deepithelialized corneas (n = 6). *P < 0.05.

Effects of Stromal Lactate in Ringer-Bathed Corneas

When 20 mM NaL was loaded into deepithelialized corneas over a 45-min period, corneas swelled more than corneas swollen in Ringer's alone (491 ± 18 μm in controls vs 558 ± 20 μm in experiments; P < 0.01; Fig. 5). A similar finding was observed with NaCl-loaded corneas (483 ± 15 vs 565 ± 20 μm; P < 0.01; Fig. 6). Upon application of silicone oil to the bare stromal surface, the loaded corneas thinned faster than did Ringer's preswollen tissues. A comparison of loaded corneas at 1.5 hr with controls at zero time demonstrated that their thickness and deswelling rates were identical. The lactate concentration in the lactate-loaded stroma was higher than in Ringer's swollen controls at zero time, but not at 1.5 hr (Fig. 7).

Effects of Stromal Lactate in Lactate-Bathed Corneas

When both loaded and bathed with 20 mM NaL or excess NaCl, deepithelialized corneas swelled to simi-
Fig. 7. Lactate concentrations measured in lactate-loaded, Ringer's-superfused stroma-endothelial tissues immediately after loading, or 1.5 hr after removal of the 20 mM lactate Ringer's from the stromal surface. Groups bearing identical superscripts are significantly different from each other \( (P < 0.05; \text{analysis of variance and Bonferroni } t\text{-test}) \).

Effects of Lactate in the Tears Bath

On the tears side, 5 mM NaL or 5 mM excess NaCl thinned corneas, compared to Ringer's bathed controls (Figs. 10, 11). Because tears-side concentrations of lactate are not elevated during corneal hypoxia, \(^{20}\) higher concentrations were not examined.

Discussion

This study demonstrates that 5 and 20 mM NaL is similar in all respects to equimolar excesses of NaCl. The immediate changes within 30 min of exposure to hypertonic solutions were not examined, but have been addressed elsewhere.\(^{21-23}\) From the aqueous side, neither agent had significant effects on the thickness of normal corneas (Figs. 1, 2), an observation which contrasted with the findings of Mishima and Hedbys.\(^{21}\) Their study clearly demonstrated the osmotic effects of 10–40 mM glucose on corneal thickness, and Wilson et al.\(^{22}\) demonstrated similar effects with 1.25% NaCl. Those investigators had used preparations bathed on one side with silicone oil, however, preventing stromal-to-tears water and solute equilibration. The corneas used here were bathed with Ringer solution on both surfaces, constituting a multicompartment system which involves fluid and solute equilibration among the stroma and tears and aqueous sides, as addressed by Klyce and Russell.\(^{23}\) In preswollen corneas with silicone oil on their deep epithelialized surface, 20 mM NaL or excess NaCl did significantly deswell corneas (Figs. 3, 4). The data suggests that lactate has no acutely deleterious effect on endothelium-mediated deturgescence.

Lactate-loaded corneas bathed in Ringer’s (Fig. 5), contained more lactate (Fig. 8) and swelled more than Ringer’s-loaded controls. Some lactate was lost
Implications for Hypoxic Corneal Edema

It appears that in vitro, and in the concentrations examined, the effects of neutral NaL are similar in all respects to excesses of NaCl, and at these concentrations, lactate has no acute toxic effect on the epithelium, endothelium, or stroma to influence corneal thickness. This contrasts with the toxic effects of neutral lactate in cardiac tissue.\footnote{6-8} Corneas loaded with NaL do swell osmotically, however. Thus, neutral lactate accumulation in the stroma can osmotically account for hypoxic edema as suggested by Klyce.\footnote{4} Although the lactate accumulation can probably explain edema, the other consequences of corneal hypoxia (morphologic changes\footnote{10-12} and acidosis\footnote{24-26}) warrant further study for their etiologies and effects on this tissue.

Key words: cornea, edema, lactate, rabbit, specular microscopy, stroma

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References


Fig. 11. In the tears side bath, a 5 mM excess of NaCl deswelled corneas compared to Ringer's-bathed controls (n = 6). *P < 0.01.

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**References**