Acidosis, alkalosis, and aqueous humor dynamics in rabbits

Theodore Krupin, Charles J. Oestrich, Jonathan Bass, Steven M. Podos,* and Bernard Becker

Systemic acidosis induced by intravenous administration of hydrochloric acid lowered intraocular pressure in unanesthetized rabbits. Aqueous humor flow was reduced by approximately 50 percent, as measured by the iodide method and as calculated from tonographic data. Outflow facility, episcleral venous pressure, plasma osmolality, blood pressure, pulse, and body temperature were not altered by systemic acidosis. Systemic alkalosis induced by intravenously administered sodium bicarbonate was associated with an increased intraocular pressure. Aqueous humor flow following systemic alkalosis was increased by approximately 100 percent, as measured by the iodide method and as calculated from tonographic data. Alkalosis was not associated with alterations in outflow facility, episcleral venous pressure, plasma osmolality, blood pressure, pulse, or rectal temperature.

Key words: acidosis, alkalosis, aqueous humor flow, blood Pco₂, rabbits.

Systemic acidosis reduces aqueous humor production and lowers intraocular pressure. The reduction of intraocular pressure is similar following either respiratory or metabolic acidosis. Part of the decrease in intraocular pressure which occurs after treatment with the carbonic anhydrase inhibitor acetazolamide and following physical activity is explained by an induced systemic acidosis.

The elevation of intraocular pressure noted in studies of rabbits during 1 hr. of systemic hyperthermia is associated with an early and transient low degree of systemic alkalosis. The purpose of the present study is to investigate further the effects of alterations of systemic blood pH on aqueous humor dynamics in rabbits.

Methods

Male albino rabbits weighing 2.5 to 3.0 kg were studied. Intraocular pressure was measured with a manometrically calibrated pneumotonograph after topical anesthesia with 0.5 percent proparacaine HCl. Tonography under similar topical anesthesia with the Berkeley electronic tonometer (Berkeley Mo-Engineering, Inc., San Leandro, Calif.) and Leeds & Northrup recorder (Leeds & Northrup, St. Louis, Mo. 63110).
Table I. Effect of intravenous HCl on intraocular pressure in 7 rabbits

<table>
<thead>
<tr>
<th>Minutes after onset of intravenous administration</th>
<th>Baseline*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Intraocular pressure (mm Hg)</td>
<td>14.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10.2 ± 0.2</td>
</tr>
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</table>

*Mean ± S.E.M.
(Significant difference from baseline, p < 0.005.)

Northrup Co., North Wales, Pa.) was also performed. Episcleral venous pressure was measured with a 3 mm. planating head attached to a force-displacement transducer and mounted on a Haag-Streit biomicroscope7 (Haag-Streit AG, Berne, Switzerland). Heparinized arterial blood samples were obtained from the central ear artery for determination of blood pH (Beckman expanded-scale pH meter; Beckman Instruments, Inc., Palo Alto, Calif.), plasma osmolality (Advanced osmometer; Advanced Instruments, Inc., Needham Heights, Mass.), and Pco2 (Model 213; Instrumentation Laboratory, Inc., Lexington, Mass.). Rectal temperature was measured with a rectal probe connected to a telethermometer (Model 43TF; Yellow Springs Instrument Co., Yellow Springs, Ohio). Blood pressure and pulse were determined over the femoral artery with a Doppler transducer assembly (Arteriosonde 1010; Hoffmann-LaRoche, Inc., Nutley, N. J.).

Intravenous solutions (39° C.) were administered through the marginal ear vein with the use of a scalp vein assembly. A 0.14N hydrochloric acid (HCl) solution was prepared with distilled water. This solution had a pH of 1.1 and an osmolality of 292 mOsm./L. An alkaline solution of 1.5 percent sodium bicarbonate (NaHCO3) was prepared with distilled water. This solution had a pH of 8.44 and an osmolality of 300 mOsm./L. The control 0.9 percent sodium chloride solution had a pH of 7.2 and an osmolality of 300 mOsm./L.

Unanesthetized rabbits were placed in a box holder. After a 30 min. adaptation interval, baseline intraocular pressure was measured, and an arterial blood specimen obtained from the right ear. The intravenous administration was started in the right ear, with all subsequent blood samples taken from the left ear. A 7 ml. amount of the HCl solution was given rapidly, followed by delivery of approximately 2.5 ml/min. over the next 60 min. (total acid load, 42.9 mEq.). The NaHCO3 and control saline solutions were delivered at a rate of 4 ml/min. for 1 hr. (total alkali load, 42.9 mEq.). Repeat intraocular pressure and blood studies were performed at 15, 30, 45 and 60 min. following the onset of intravenous administration. Rectal temperature was monitored. Tonography and episcleral venous pressure measurements were obtained before and 1 hr. after the intravenous administration HCl and NaHCO3.

Aqueous humor flow (F) was calculated from the tonographic data using the equation F = (Po — Pv)C where Po is the intraocular pressure, Pv the episcleral venous pressure, and C the outflow facility.

The rate of aqueous humor flow was determined by Becker’s method using two isotopes of iodide.* The rabbits were given 0.5 mg/kg. sodium iodide (127I) intraperitoneally 18 to 24 hr. before the experiment to saturate thyroidal and other iodide binding sites. 131I was given (45 μCi), one third intravenously and two thirds intraperitoneally, at 3 hr. before the intravenous administration of either HCl, NaHCO3, or NaCl. 125I was given (45 μCi) 15 min. after the beginning of intravenous administration, one third intravenously and two thirds intraperitoneally. Anterior chamber paracentesis was accomplished under topical anesthesia with 0.5 percent proparacaine hydrochloride. Paracentesis of the right eye was performed at 30 min. and of the left eye at 60 min. following delivery of 125I. Simultaneous heparinized blood samples were obtained. Aqueous humor and plasma 131I and 125I radioactivity were measured simultaneously in a Packard scintillation counter (Model 3375; Packard Instrument Co., Inc., Downer’s Grove, Ill.), with the use of an external standard.

Mathematical formulations were performed with the equation of Kinsey,4 disregarding the contribution of the posterior chamber.

\[
\frac{dC_a}{dt} = k_{iMa} (\alpha C_p - C_a) - k_{a} C_a
\]

where \(\frac{dC_a}{dt}\) = the rate of change in concentration of iodide in the anterior chamber aqueous humor

\(k_{iMa}\) = the coefficient of diffusional exchange of iodide between anterior chamber aqueous humor and plasma (min\(^{-1}\))

\(k_{a}\) = fraction of anterior chamber aqueous humor leaving the eye by flow (min\(^{-1}\))

\(\alpha\) = the Donnan factor for iodide (1.05)

\(C_a\) = aqueous humor iodide concentration (as a fraction of the concentration in the plasma water)

\(C_p\) = plasma iodide concentration = 1.0

*Significant difference from baseline, p < 0.005.
If \( k_{\text{out}} = k_{\text{in}} + k_{\text{fa}} \), then equation 1 can be written:

\[
\frac{dC_a}{dt} = k_{\text{in}} C_p - k_{\text{out}} C_a \tag{2}
\]

At steady state,

\[
\frac{dC_a}{dt} = 0 \text{ and } C_a = C_a^\infty
\]

where \( C_a^\infty \) is the steady-state concentration in the aqueous humor and

\[
k_{\text{in}} = \frac{k_{\text{in}} C_a^\infty}{C_p}
\]

Substituting in equation 2:

\[
\frac{dC_a}{dt} = k_{\text{in}} (C_a^\infty - C_a) \tag{4}
\]

and by integration:

\[
2.3 \log (C_a^\infty - C_a) = -k_{\text{out}} \cdot t \tag{6}
\]

\( k_{\text{out}} \) was graphically estimated by plotting \( \log (C_a^\infty - C_a) \) against time in minutes for the data obtained from the paracentesis of each rabbit. This provided a straight line of slope, \(-k_{\text{out}}/2.3\).

The aqueous humor and plasma \({}_3\text{H}I\) values were constant for the two paracenteses of each rabbit following the administration of HC1, NaHCO3, and NaCl. The steady state allowed calculation of \( k_{\text{in}} \) from equation 3; then the flow coefficient, \( K_{\text{out}} K_{\text{fa}} \).

**Results**

**Acidosis.** The intravenous administration of HC1 resulted in a significant (\( p < 0.005 \)) reduction in intraocular pressure and blood pH as compared to baseline values (Table I). Most of the alteration in intraocular pressure and blood pH occurred within the first 30 min. after initiation of the injection. These parameters returned to original levels 1 hr. after the intravenous administration was discontinued. Rectal temperature, blood pressure, and pulse were not altered during the experiment. The intravenous delivery of HC1 did not change plasma osmolality, since the baseline value of 301.3 ± 4.5 mOsm./L. (mean ± S.E.M.) was not significantly different (\( p > 0.8 \)) from 303.5 ± 6.0 mOsm./L. at 1 hr. following acid treatment. Tonography confirmed the reduction in intraocular pressure without demonstrating any alteration in outflow facility. Baseline tonograms showed an intraocular pressure of 17.2 ± 0.8 mm. Hg and an outflow facility of 0.30 ± 0.02 \( \mu l/min./mm. \) Hg. Repeat tonograms 1 hr. after acidosis demonstrated a decrease in intraocular pressure to 14.0 ± 0.8 mm. Hg and an unchanged outflow facility of 0.29 ± 0.03 \( \mu l/min./mm. \) Hg. This reduction in intraocular pressure was not associated with a change in mean episcleral venous pressure (11 mm. Hg). Estimated aqueous humor flow was thus decreased by 47 percent.

**Table II. Iodide turnover in 7 rabbits treated with intravenous HCl**

<table>
<thead>
<tr>
<th></th>
<th>( k_{\text{out}} ) (min⁻¹)</th>
<th>( C_a^\infty ) (Cp)</th>
<th>( k_{\text{in}} ) (min⁻¹)</th>
<th>( k_{\text{fa}} ) (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.015</td>
<td>0.47</td>
<td>0.007</td>
<td>0.008</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0.002</td>
<td>0.02</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*See text for times of iodide and HCl (0.1N, pH 1.1, 292 mOsm./L.) administration.

**Table III. Iodide turnover in 7 rabbits treated with intravenous NaCl**

<table>
<thead>
<tr>
<th></th>
<th>( k_{\text{out}} ) (min⁻¹)</th>
<th>( C_a^\infty ) (Cp)</th>
<th>( k_{\text{in}} ) (min⁻¹)</th>
<th>( k_{\text{fa}} ) (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.034</td>
<td>0.50</td>
<td>0.015</td>
<td>0.018</td>
</tr>
<tr>
<td>S.E.M.</td>
<td>0.002</td>
<td>0.02</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*See text for times of iodide and NaCl (0.9%, pH 7.2, 300 mOsm./L.) administration.
Table IV. Effect of intravenous NaHCO3 on intraocular pressure in 9 rabbits

<table>
<thead>
<tr>
<th>Minutes after onset of intravenous administration</th>
<th>Baseline*</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraocular pressure (mm. Hg)</td>
<td>15.2 ± 0.4</td>
<td>20.2†</td>
<td>20.5†</td>
<td>21.1†</td>
<td>22.7†</td>
</tr>
<tr>
<td>Blood pH</td>
<td>7.50 ± 0.02</td>
<td>7.64†</td>
<td>7.71†</td>
<td>7.76†</td>
<td>7.77†</td>
</tr>
</tbody>
</table>

*Mean ± S.E.M.  
†Significant difference from baseline, p < 0.005.

Table V. Iodide turnover in 7 rabbits treated with intravenous NaHCO3

<table>
<thead>
<tr>
<th>kout (min⁻¹)</th>
<th>Cαα (min⁻¹)</th>
<th>kdep (min⁻¹)</th>
<th>kin (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.054</td>
<td>0.025</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>0.070</td>
<td>0.029</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>0.072</td>
<td>0.036</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>0.061</td>
<td>0.020</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>0.074</td>
<td>0.042</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>0.069</td>
<td>0.038</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>0.069</td>
<td>0.012</td>
<td>0.037</td>
<td></td>
</tr>
</tbody>
</table>

Mean 0.067 0.049 0.012 0.035 0.003 0.003 0.002

*See text for times of iodide and NaHCO3 (1.5%, pH 8.44, 300 mOs/L) administration.

reduction in kin when compared to the aqueous humor flow coefficient of 0.018 ± 0.001 following control NaCl infusion (Table III). Control animals demonstrated no alterations of intraocular pressure, blood pH, or plasma osmolality. The reduction in kν was highly significant (p < 0.001).

Alkalosis. An increase in intraocular pressure and blood pH occurred following intravenous administration of NaHCO3 (Table IV). The greatest change occurred within the first 15 min. of intravenous administration. The increases were highly significant (p < 0.005) at all times tested. Baseline total CO2 was 19.9 mM/L in five rabbits. This increased to 45.4 mM/L after 1 hr. of alkali treatment. Intraocular pressure and blood pH returned to pretreatment levels 60 min. after the intravenous solution was stopped. The effect of alkalosis on intraocular pressure was not associated with alterations of systemic blood pressure, temperature, or pulse. Plasma osmolality was unchanged from a baseline mean value of 292.2 ± 2.4 mOsm/L to a level of 293.8 ± 4.1 at 1 hr. following the intravenous administration of NaHCO3. Episceral venous pressure averaged 11 mm. Hg before and 1 hr. following alkalosis. Baseline tonograms demonstrated an intraocular pressure of 17.1 ± 1.3 mm. Hg and an outflow facility of 0.31 ± 0.03. At 1 hr. following alkalosis intraocular pressure was 21.2 ± 1.3 mm. Hg and outflow facility was 0.34 ± 0.03. This represented an 84 percent increase in tonographically measured aqueous humor flow.

The aqueous humor flow coefficient as determined by the iodide method following systemic alkalosis was 0.035 ± 0.002 (Table V). This represented a significant (p < 0.001) increase over the flow coefficient in control animals and a 94 percent increase in the apparent rate of aqueous humor formation.

Discussion

The results of these experiments support the view that in rabbits uncompensated metabolic acidosis (decreased pH and total CO2 with unchanged Pco2) lowers intraocular pressure by decreasing aqueous humor flow. In unanesthetized animals a reduction in blood pH of 0.16 is associated with an apparent 56 percent reduction in aqueous humor flow coefficient as determined by the iodide method. Aqueous humor flow as calculated from the tonographic data was also reduced by approximately 50 percent following systemic acidosis. The level of acidosis in the present study is of the same magnitude as that seen immediately following exercise. However, exercise which also increases plasma osmolality results in a greater reduction of intraocular pressure. The decreases of intraocular pressure and aqueous humor flow following acidosis are similar to the decreases observed after acetazolamide administration.

The systemic acidosis induced by acetazolamide is of lesser magnitude than produced in the present study. The relationship be-
between systemic acid-based changes and acetazolamide is under investigation.

More interesting, the present study demonstrates that metabolic alkalosis elevates intraocular pressure in association with an increase in aqueous humor flow. This increase in the rate of aqueous humor formation is 94 percent by the iodide method and 84 percent from tonographic data. These changes of alkalosis are similar to those observed following hyperthermia in rabbits. A rapid increase in body temperature of 1.6° C. elevates aqueous humor flow by 126 percent. In the present study, rectal temperature was unchanged following systemic alkalosis. The rise in intraocular pressure with metabolic alkalosis disagrees with the lack of effect previously reported. However, the magnitude (pH and total CO₂) and the rate of alkalosis is considerably greater in the present study. Also, the condition of metabolic alkalosis is partially compensated by respiratory mechanisms, with a resultant increase in PCO₂. Recent studies demonstrate that increasing PCO₂ by primary respiratory methods elevates intraocular pressure. In addition to the present finding of increased aqueous humor flow, elevated PCO₂ increases uveal blood flow.

Additional studies are in progress investigating the mechanism by which blood pH alters aqueous humor production. Possible explanations for the effects of acid-base changes include alterations of ciliary body active membrane transport, central nervous system and neurologic mechanisms, permeability changes, and/or chemical reactions.

REFERENCES