Rat Muscle Cell pH After Ouabain Administration[1] (34575)

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Measurements of cell acid-base status by a variety of techniques and in a number of species have frequently revealed higher values for cell pH than can be accounted for if intracellular hydrogen ion concentration is assumed to be determined solely by a Donnan distribution (1). It has been suggested that such a disequilibrium between intra- and extracellular hydrogen ion concentrations may indicate the existence of an intracellular mechanism for removal of protons by active transport from cell to extracellular fluid (2). That such transport, in a system dependent upon cardiac glycoside-sensitive ATPase, may be a mechanism by which cell pH is regulated, and disequilibrium hydrogen ion concentrations maintained, was tested in the present experiments by estimation of muscle cell pH in rats after ouabain administration.

Methods. Male Sprague-Dawley rats weighing 400–500 g were used in all experiments. After the animals had been anesthetized with 5 % pentobarbital sodium (1 ml/kg given intraperitoneally) the neck vessels were exposed and Teflon cannulae placed in the left external jugular vein and the left common carotid artery. The arterial cannula was connected to a Stratham strain gauge, and blood pressure was monitored on an Electronics for Medicine recorder. After exposure through the abdominal skin, an approximately 1-cm incision was made in the rectus sheath, and the Teflon-covered tip of a Radiometer PCO_2 electrode was placed against the exposed muscle. Abdominal skin was then drawn up and sutured tightly around the shaft of the elctrode, which was secured by clamps and connected to a Radiometer pH meter previously calibrated for PCO_2 with the same electrode against several CO_2–O_2 gas mixtures. After the intravenous injection of tubocurarine chloride (0.09 mg) animals were artificially ventilated with 100 % O_2 through a tracheostomy tube by a Phipps and Bird small animal respirator. Respirator stroke volume and rate were varied as necessary to maintain stable values for PCO_2.

After a period of equilibration and stabilization of PCO_2 each animal was given intravenously, at 0.1 ml/min, 0.6 ml/100 g body weight of one of the following: phosphate-buffered saline; phosphate-buffered saline containing ouabain, 0.25 mg/ml (0.15 mg/100 g); phosphate-buffered saline containing ouabain, 0.25 mg/ml, and NH_4Cl, 44.7 mg/ml (0.5 meq/100 g). Carrier-free $H_2{}^{35}SO_4$, 25 μCi/kg, was given intravenously 60 min before the end of each experiment. Five to ten minutes after the close of each infusion the abdomen was opened, and a blood sample was obtained from the inferior vena cava in an oiled, heparinized syringe for pH, plasma CO_2 content, and electrolyte analyses. The animal was then sacrificed by exsanguination as hind limb muscle tissue was quickly frozen *in situ* with liquid nitrogen. Muscle samples of 150–200 mg were cut out and weighed in the frozen state prior to analysis for CO_2 content. Larger muscle samples were taken for analysis of water content and electrolyte composition.

Total acid-extractable CO_2 was determined on frozen muscle tissue by a modification (3) of the microdiffusion technique of Conway (4) as adapted by Anderson and Mudge (5). The pH of whole blood was determined anaerobically at 37° in a Radiometer pH

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TABLE I. Effects of Ouabain on Cell Bicarbonate and pH.[a]

	NaCl	Ouabain	Ouabain–NH_4Cl
No. of animals	8	7	7
Plasma			
$[K^+]$ meq/liter	4.6 ± 0.4	10.4 ± 0.7	10.1 ± 1.0
$[HCO_3^-]$ meq/liter	27.9 ± 1.2	25.9 ± 0.3	20.1 ± 2.2
pH	7.46	7.42	7.30
$[H^+]$ neq/liter	34.0 ± 2.6	37.4 ± 3.8	49.9 ± 4.5
PCO_2, mm Hg	39.7 ± 3.8	40.5 ± 3.9	41.9 ± 4.6
Muscle			
Total H_2O, ml/100 g dry wt	315.0 ± 6.6	325.9 ± 5.0	322.7 ± 6.0
EC H_2O, ml/100 g dry wt	34.8 ± 3.8	34.4 ± 4.5	40.0 ± 3.2
IC H_2O, ml/100 g dry wt	280.3 ± 2.9	291.5 ± 6.2	282.8 ± 6.8
Total CO_2, mmole/kg wet wt	10.9 ± 0.3	10.0 ± 0.5	9.9 ± 0.9
$[HCO_3^-]$ meq/liter cell H_2O	10.8 ± 0.5	9.7 ± 0.8	10.1 ± 0.9
$[K^+]$ meq/liter cell H_2O	157.3 ± 5.5	149.6 ± 3.8	139.1 ±30.4
pH	6.99	6.94	6.94
$[H^+]$ neq/liter cell H_2O	102.4 ± 3.7	116.5 ±12.5	115.6 ± 8.9

[a] Values are expressed as mean ± SD.

meter with water-jacketed microelectrodes. Total CO_2 content of plasma was measured by standard manometric technique. Total muscle water was determined by drying whole wet tissue at 95° for approximately 18 hr. Potassium was measured by flame photometry on plasma, and on ashed muscle samples extracted in 1 *N* H_2SO_4. Weighed muscle samples were ground in 10% trichloroacetic acid with a Servall tissue homogenizer, and, after centrifugation, sulfate was precipitated from a portion of the supernate with benzidine dihydrochloride. Sufficient carrier sulfate was added to give a thickness of 25 mg/cm^2 when the precipitate was transferred in 95 % alcohol to 2.25-cm^2 ruled, paraffin-rimmed filter paper disks on suction funnels. After vacuum drying for 1 hr the precipitate mats were counted in a Nuclear-Chicago flow counter for 20,000 counts at an average rate of 9.2 times background. Radiosulfate was similarly precipitated and counted in plasma samples deproteinized with trichloroacetic acid.

Total tissue bicarbonate was calculated from acid-extractable CO_2 content by subtraction of the dissolved CO_2 of extracellular and cell water. Intracellular bicarbonate was determined by subtraction of extracellular bicarbonate, using the apparent volume of distribution of radiosulfate as a measure of extracellular space. Intracellular pH was calculated from the dissolved CO_2 and the intracellular bicarbonate, using the Henderson-Hasselbalch equation and a value for pK'_1 of 6.1 (6). Solubility coefficients for CO_2 in plasma, interstitial and cell water were taken as 0.553, 0.540 and 0.590 cc/g, respectively (6, 7). A Donnan factor of 0.95 was assumed for bicarbonate (8), of 0.92 for potassium (9), and 0.90 for sulfate (10). The calculations also include an assumed plasma water content of 93 %.

Results. Although occasional transient abnormalities of pulse were observed during ouabain administration, animals appeared to tolerate the procedure well. The average change in mean blood pressure for all experiments was —8.2 ± 15.7 (SD) mm Hg; and for those in which ouabain was given, —17.4 ± 17.8. The mean differences of terminal PCO_2 meter readings from venous blood PCO_2, calculated from pH and CO_2 content, were: —2.5 ± 2.5 and + 2.5 ± 1.8 mm Hg. The mean maximum range of meter PCO_2 variation during all experiments was 4.1 ± 1.3 mm Hg.

Data from the three experimental groups are shown in Table I. For control animals the mean total CO_2 content for muscle was 10.9

mmole/kg wet weight. Calculated intracellular bicarbonate concentration was 10.8 meq/liter cell water, pH 6.99, and hydrogen ion concentration 102 neq/liter cell water. Mean intracellular potassium concentration was 157.3 meq/liter.

Animals given ouabain alone did not show an appreciable difference in plasma hydrogen ion or bicarbonate concentrations when compared with saline-treated controls. Mean intracellular potassium concentration was significantly[2] lower and extracellular potassium significantly higher than in control animals, and the mean intra- to extracellular potassium concentration ratio was approximately one half that of controls. Calculated cell pH was similar in saline- and ouabain-treated animals, and there was not a significant difference between their mean hydrogen ion concentrations.

Animals given ammonium chloride with ouabain showed a 47 % higher mean plasma hydrogen ion concentration than saline controls without appreciable difference in PCO_2. Like animals treated with ouabain alone, the group receiving ammonium chloride with ouabain had a mean intracellular potassium concentration that was significantly lower, and an extracellular potassium that was higher than controls while cell hydrogen ion concentration was not significantly different.

Discussion. Evidence for cardiac glycoside inhibition of a cellular mechanism for maintaining lower than equilibrium intracellular hydrogen ion concentration was not found in the present study on rat muscle. Although mean calculated intracellular hydrogen ion concentration was slightly higher in ouabain-treated animals than in the control group, the difference was not statistically significant. In view of the changes in cell and extracellular potassium concentrations, it seems unlikely that the relative stability of intracellular pH in ouabain-treated animals simply reflects a concentration of drug at muscle insufficient to materially affect electrolyte transport. On the other hand, the fact that mean cell hydrogen ion concentration was slightly higher in ouabain-treated than in control animals, raises the possibility that an effect of ouabain on cell pH was present, but insufficient to cause clear differences in mean cell hydrogen ion concentration when the groups were compared statistically. The experiment in which ouabain administration was combined with metabolic acidosis does not support this interpretation, however. Under the circumstances of partial inhibition of outward hydrogen ion transport by the cell, increases in extracellular hydrogen ion concentration would be expected to be reflected by further increases in cell hydrogen. In animals given ouabain and ammonium chloride, mean cell hydrogen ion concentration was not significantly different from that of the group given ouabain alone, nor from that of normal controls.

Recent microelectrode studies (11) have raised basic questions about the true magnitude of pH gradients across the normal muscle cell membrane, but the present experiments do not bear critically on this point. They have been interpreted only as evidence in support of the view that normal cell pH is not maintained through hydrogen transport by ouabain-sensitive systems; they do not exclude other forms of transport, specialized intracellular buffering, or equilibrium hydrogen ion distribution.

Summary. Rats were given intravenous ouabain, alone or with ammonium chloride, and muscle cell bicarbonate concentration and pH were calculated from total tissue CO_2 content. In spite of clear changes in intra- and extracellular potassium concentrations, cell pH was not materially affected by these procedures. The data suggest that normal intracellular pH in rat muscle is not dependent upon outward transport of hydrogen ion by ouabain-sensitive systems.

[2] In the description of data a difference between group means has been said to be significant when the t test gives a value for $p < .01$.

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