Potassium and Its Effect on Glycogen in the Dog Heart

By Arthur W. Merrick, Ph.D.

There are surprisingly few reports in the literature having to do with the inter-relationship of potassium and cardiac glycogen. The experiments described in this paper were designed to determine what changes, if any, might occur in the acid-soluble and acid-insoluble glycogen fractions of the dog heart when (a) isotonic KCl continuously was infused into a normal, anesthetized animal; (b) slices were exposed to media containing variable concentrations of potassium; and (c) a homogenate was incubated for varying periods of time in an "intracellular" glucose-containing fluid medium similar to that suggested by Hastings.

Methods

Infusion of Potassium Chloride

Ten mongrel dogs were anesthetized with sodium pentobarbital (32.5 mg./Kg.), and the right femoral vein was exposed for infusion of isotonic potassium chloride. Prior to and during infusion electrocardiograms were recorded, and blood samples were drawn for serum potassium determinations. The infusion rate was based on the weight of the animal, and at the appearance of bizarre complexes in the electrocardiogram at an average of 2 hours, the heart was quickly excised. One piece of tissue (ca. 500 mg.) from each chamber was removed and analyzed for total glycogen. A second piece of tissue from each chamber of the heart was quick-frozen immediately between blocks of carbon dioxide ice and later analyzed for acid-soluble glycogen according to a method described by Bloom, Lewis, Schumpert and Shen. Total glycogen and acid-soluble glycogen quantitatively were determined by the anthrone procedure suggested by Seifter, Dayton, Novic, and Muntwyler. The difference between the 2 has been described as the acid-insoluble fraction. The concentration of potassium in the serum was determined by the Perkin-Elmer flame photometer using the internal standard procedure. Control values were obtained from 10 dogs subjected to anesthesia for approximately 2 hours; electrocardiographic recordings and serum potassium analyses were made from these animals.

Incubation of Heart Slices

The heart was removed from each of 10 mongrel dogs previously anesthetized with sodium pentobarbital. A section of the left ventricle, approximately 2 cm. square and 1 cm. thick, was removed from 3 cm. below the origin of the left anterior descending coronary artery. The procedure for proper slicing and handling of the tissue has been described elsewhere. A piece of tissue weighing approximately 150 mg. initially was cut from the ventricular section and immediately frozen in dry ice. The glycogen content of this served as the control "before slicing."

Tissue slices of approximately 50 mg. in weight were obtained from the remaining ventricular section and placed in standard Warburg flasks. These flasks contained 2.8 ml. of a solution whose final concentration was 2 per cent glucose and 120 mM NaCl, 4.8 mM KCl, 2.6 mM CaCl₂, 1.2 mM MgSO₄ • 7H₂O, 7H₂O, and 15.6 mM Na₂HPO₄ • 7H₂O with a pH of 7.35. Subsequent to appropriate gassing of the manometers and flasks with 100 per cent O₂, the manometers were equilibrated and the flasks shaken at the rate of 115/min. for 2 hours. Oxygen consumption was recorded every 15 minutes during this 2-hour shaking period. Aside from the manometer designated as the thermobarometer, 4 of the flask side arms contained 0.2 ml. of the basic solution.

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Table 1

<table>
<thead>
<tr>
<th>Glycogen Fraction</th>
<th>Control (mg./100 Gm.)</th>
<th>Potassium-infused (mg./100 Gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RA</td>
<td>LA</td>
</tr>
<tr>
<td>Acid-soluble</td>
<td>588±60t</td>
<td>801±73*</td>
</tr>
<tr>
<td>Acid-insoluble</td>
<td>84±38</td>
<td>144±44</td>
</tr>
<tr>
<td>Total</td>
<td>672±45</td>
<td>945±78</td>
</tr>
</tbody>
</table>

*Each figure represents the mean of 10 animals.
†SEM.

incubating solution. The side arms of the remaining 12 flasks contained 0.2 ml. of this solution plus a variable concentration of KCl. When this 0.2 ml. was tipped into the flask, the final concentration of the basic incubating medium in each of 4 flasks was 3, 6, and 9 mM of potassium. The glycogen content of the slices in the flasks, into which the basic incubating solution only had been tipped, served as the control “after incubation.”

It was necessary to determine what effects the slicing procedure would have on ventricular glycogen. Consequently, slices having a combined weight of approximately 150 mg. were obtained from the remaining piece of ventricle immediately after appropriate treatment of the slices for the Warburg flasks. This tissue mass was quick-frozen in dry ice, and the glycogen content served as the control “after slicing.”

The procedures for the glycogen fraction analyses were carried out by methods already discussed.6

Tissue Homogenate

Subsequent to anesthesia the hearts were removed from 10 mongrel dogs. A section from the left ventricle, similar to that described previously, was rapidly excised and homogenized in an ice-cold mixture of 2 ml. of the “intracellular” medium and 0.2 ml. of a 5.4 per cent glucose solution at a pH of 7.22. The total millimolarity of the potassium in the “intracellular” medium was 109 resulting from 69 mM KCl and 40 mM KHCO3. The tissue homogenate was incubated, in appropriately gassed (100 per cent O2) Warburg flasks at 37 C, for intervals of 10 minutes up to a maximum of 50 minutes and subsequently analyzed for glycogen.

Results

Infusion

It is apparent from the data (table 1) that hyperpotassemia had little effect on the glycogen fractions of the dog heart. There was no significant difference between any of the 4 chambers. There was a less than 6 per cent difference in the mean total of the control and experimental groups. The experimental mean of the acid-soluble glycogen fraction for the combined chambers of the heart varied less than 7 per cent from normal, and the mean of the acid-insoluble form showed approximately a 9 per cent difference. No trend or tendency to change was observed. In the control group the acid-soluble glycogen fraction amounted to approximately 85 to 88 percent of the total available glycogen in the various heart chambers. In the experimental animals the percentage values were of the same order of magnitude although the acid-soluble form in the left ventricle decreased to 79 per cent. Potassium was known to be high, as indicated by the analysis of the serum level of this ion (average of 9.5 mEq./L.) and from the regular sequence of events seen in the electrocardiograms with progressive hyperpotassemia.3

Slices

Slightly less than one-half (47 per cent) of the absolute amount of acid-soluble glycogen was lost during the slicing process. The acid-insoluble or stable form decreased 43 percent (table 2), and this may be of considerable significance to the tissue. Subsequent to incubation the controls exhibited an additional absolute loss in the acid-soluble glycogen fraction. In all phases of controls and experimental specimens, the absolute percentage of one glycogen fraction to the other did not vary by more than 6 per cent; therefore, it is unlikely that any interconversion of glycogen might have occurred. A statistical comparison between each of the 3 experimental groups and the control “after incubation” revealed that
significant glycogenolysis occurred only in the acid-soluble component when the cardiac slices were incubated in 9 mM of potassium (P = .002). No other significant increase or decrease was observed in either of the 2 glycogen forms.

Homogenate

When a portion of the left ventricle of the heart was homogenized and then incubated in a simulated "intracellular" medium for 10-minute intervals to a maximum of 50 minutes (table 3), progressive glycogenolysis was observed in both glycogen fractions. The loss was most pronounced and rapid in the acid-soluble form. Ten minutes after incubation this labile fraction was reduced significantly, and the degradation continued for the duration of the experiment. The more stable glycogen fraction similarly was degraded but at a slower and more uniform rate. A statistically significant change (P = .018) was not observed until 50 minutes after incubation was initiated.

Discussion

The infusion experiments indicate that hyperpotassemia does not alter the level of glycogen in the dog heart. Silvette, Britton, and Kline showed that either glycogenolysis or inhibition of the glycogenic processes in the rat and cat heart and liver does occur subsequent to an intraperitoneal injection of a sublethal dose of potassium chloride. A subtoxic dose of potassium chloride infused into a dog produced a significant loss in liver glycogen. Seibert and Huggins were of the opinion the high serum potassium concentration was responsible for the liver glycogenolysis and not the liberation of epinephrine from the adrenals. Wajzer reported that potassium chloride in excess would deplete the "lyoglycogen" (acid-soluble) fraction in muscle. Glycogenolysis of the "desmoglycogen" (acid-insoluble) form could occur during the initial degradation of the free form or somewhat later on, but only part of this fraction was split. Poppen, Green, and Wrenn in a histochemical study suggested a potassium-glycogen relationship did exist in that large amounts of intracellular potassium were found in glycogen-rich zones in both the human and lamb heart and liver.

The termination of this in vivo series of experiments supports the following hypotheses: (a) cardiac glycogen synthesis is not enhanced in a potassium-rich environment, nor (b) is the glycogen present in the heart degraded. The normal glycogen levels in the chambers of the dog heart were reported in previous papers and the ventricular values corroborated the finding of Cruickshank.

A considerable number of in vitro glycogen experiments have been done utilizing tissue slices, and the interpretation of the results might be described as dissident. Variability may be expected, of course, because the number of new parameters created in an artificial environment are tremendous. The acid-soluble glycogen of dog ventricular slices incubated in variable potassium concentrations showed a decrease as the molarity of the potassium increased. Significant degrading of this form

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**Table 2**

Effects of Potassium on Glycogen Content of Dog Ventricular Slices*

<table>
<thead>
<tr>
<th>Glycogen (mg./100 Gm.)</th>
<th>Control before slicing</th>
<th>Control after slicing</th>
<th>Control after incubation</th>
<th>Potassium experimental specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-soluble</td>
<td>566±39.3</td>
<td>296±54.9</td>
<td>241±34.5</td>
<td>234±23.2</td>
</tr>
<tr>
<td>Acid-insoluble</td>
<td>49±2.5</td>
<td>26±8.9</td>
<td>39±6.5</td>
<td>30±4.8</td>
</tr>
<tr>
<td>Total</td>
<td>615</td>
<td>324</td>
<td>267</td>
<td>273</td>
</tr>
</tbody>
</table>

*Each figure represents the mean of 10 animals.

†SEM.

†Statistical comparison (P = .002).
Table 3

<table>
<thead>
<tr>
<th>Glycogen</th>
<th>Incubation time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg./100 Gm.)</td>
<td>0</td>
</tr>
<tr>
<td>Acid-soluble</td>
<td>363±20.8t</td>
</tr>
<tr>
<td>Acid-insoluble</td>
<td>144± 9.3</td>
</tr>
<tr>
<td>Total</td>
<td>507</td>
</tr>
</tbody>
</table>

*Each figure represents the mean of 10 animals.
†SEM.

was not noted, however, until the medium contained 9 mM of potassium. Similarly, homogenized and disrupted cardiac cells rapidly lost glycogen during incubation in a potassium medium. The loss was immediate and continuous in the acid-soluble fraction. Degradation in the acid-insoluble form was gradual and not significantly changed for approximately 50 minutes.

The results may be compared both favorably and unfavorably with some previous work. In the latter, rat heart slices were incubated in a medium containing a relatively high concentration of glucose, some sorbitol, a small amount of phosphate buffer, and variable amounts of potassium. Rat heart slices in media of this nature synthesized acid-soluble glycogen providing the potassium concentration was maintained at approximately 12 to 15 mM or less. No synthesis was observed above this millimolarity of potassium. The dog ventricular slices and homogenates were incubated in media of variable potassium composition, and there was no synthesis of either glycogen fraction. Rat heart slices were incubated in media quite different in composition from those in which dog ventricular slices or homogenates were incubated. These results also may be compared to those obtained with rat liver slices. The latter responded favorably to a potassium environment and apparently stimulated the glycogenic processes. Although no investigation of potassium was involved, Meyer, Russell, Platner, Purdy, and Westfall reported glycogen loss in dog heart homogenates even though adequate glucose and oxygen were available. Some synthesis of the acid-soluble glycogen form was obtained from rat heart homogenates but only when sufficient glucose and oxygen were present. If either of the latter were low, glycogenolysis occurred.

There are those investigators who believe a medium low in potassium is best for glycogen synthesis and that high potassium either inhibits this process or stimulates the glycogenolytic mechanism. These groups used either rat diaphragm, liver, or heart slices. The media and pH levels were not the same except for the fact that glucose was present. On the other hand, there are those who feel a potassium-rich medium is best for glycogen synthesis from glucose, although Deane and his group observed this effect only at the periphery of the slice and not in the interior. The glycogen synthesis in a potassium medium reportedly is maintained at the expense of the glycolytic pathway and not the hexose monophosphate shunt. These groups used rat diaphragm, liver, or kidney slices, rabbit liver slices, or guinea pig cortical slices. The media and pH levels were variable. Synthesis of glycogen in rabbit liver slices occurred in a Ringer's solution containing glucose and having a relatively high pH. Leupin and Verzar, investigating glycogen changes in diaphragm tissue bathed in a Ringer's-glucose solution, suggested glycogen formation and potassium uptake were "coupled" in some way.

Intact dog myocardial cells have a moderately high glycogen level in comparison with those of many other species of animals. In an in situ situation the cells exhibited a strong resistance to change, and although severe electrolyte changes were produced by induced serum hyperpotassemia, the condition had little or no effect on cellular cardiac glycogen.
POTASSIUM EFFECT ON HEART

In artificial situations such as those produced by the in vitro experiments, the ability of the cells to maintain complete homeostasis was lost although efforts were made to duplicate an ideal cellular environment. As the internal milieu of the cells was further disrupted by homogenization, glycogen was degraded to a greater extent, and glycogenolysis was increased further by creating an imbalance in the ionic environment. The data obtained from slices and homogenates suggest that a potassium medium either inhibits glycogen synthesis or initiates glycogenolysis in dog myocardial tissue.

Summary

Potassium chloride, infused into 10 anesthetized dogs until severe electrical changes were observed in the heart (electrocardiogram) and serum potassium was elevated (9.5 mEq./L.), had no apparent effect on the glycogen content of any of the 4 heart chambers. Dog ventricular slices did not tolerate a medium high in potassium. Glycogenolysis was noted when the slices were incubated in a Krebs-Ringer buffered phosphate glucose medium containing 9 mil of potassium. The glycogen loss was confined to the acid-soluble form of the polysaccharide. A homogenate of dog myocardium, incubated in a simulated "intracellular" medium for up to 50 minutes, rapidly and continuously lost acid-soluble glycogen. The acid-insoluble fraction was not degraded until the homogenate was incubated for 50 minutes.

Acknowledgment

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References


Erratum

Volume 8, page 821, table 1: aortic O₂ should be corrected to read (µl. O₂/hr./Gm. wet weight).
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