EFFECT OF POTASSIUM AND HYPO镁NAGNESEMIA ON INSULIN IN THE BOVINE¹

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SUMMARY

Grass tetany in cattle has been associated with the consumption of early spring forages high in potassium (K) and low in magnesium (Mg). Alterations in serum Mg and K may affect intermediary carbohydrate metabolism, resulting in hypoglycemia and ketosis that often accompany grass tetany. We investigated these interrelationships by infusing potassium chloride (KCl) intravenously in normal (plasma Mg > 2.1 mg/100 ml) and Mg-deficient (plasma Mg < .7 mg/100 ml) 9-month-old Holstein bull calves and intraruminally into nonpregnant, nonlactating Holstein cows. Plasma levels of both K and immunoreactive insulin (IRI) were elevated (P<.01) by 1.14, 2, and 3% KCl (51, 64, and 135 mg K/kg) in calves and by 550 g KCl (440 mg K/kg body weight) in cows. Plasma K was lower (P<.01) and IRI higher (P<.01) in Mg-deficient calves than in normal calves during 2% KCl infusion. Elevated plasma K and IRI were accompanied by lower plasma glucose in normal calves (P<.05) and cows (P<.001). Elevated plasma glucose (P<.05) in Mg-deficient calves may have resulted from stimulation of glucagon secretion by abnormally high insulin levels. These results suggest that prolonged elevation of K and insulin in ruminants could lead to a series of metabolic disturbances that may play an important role in the etiology of grass tetany.

(Key Words: Potassium, Insulin, Hypomagnesemia, Bovine, Glucose.)

INTRODUCTION

Grass tetany has become one of the major metabolic disorders of cattle in many areas of the world (Grunes et al., 1970; Molloy, 1971). Incidence of grass tetany is greater in late winter and early spring (Kemp, 1960), when rapidly growing grass contains low magnesium (Mg) and high potassium (K) concentrations (Metson et al., 1966). Alterations in serum Mg (Madsen et al., 1975; Aikawa, 1960) and K (Durlock, 1971) may affect intermediary carbohydrate metabolism, since the tetany syndrome has included hypoglycemia and ketosis (Grunes et al., 1970).

Intravenous K infusion markedly increased insulin secretion in dogs (Hiatt et al., 1972). Insulin secretion by isolated perfused rat and rabbit pancreas was also increased by elevated K ion concentration but inhibited by Mg ions (Grodsky and Bennett, 1966; Howell and Taylor, 1968; Hales and Milner, 1968). Both of these responses were independent of glucose concentration.

These observations suggest that high K and low Mg concentrations, characteristic of many rapidly growing early spring forages, may contribute to the onset of grass tetany by altering intermediary carbohydrate metabolism. Our objective was to determine the effects of elevated plasma K and hypomagnesemia on plasma insulin and glucose in the bovine.

EXPERIMENTAL PROCEDURE

A series of three experiments was performed with Holstein bull calves and mature nonpregnant, nonlactating Holstein cows.

Experiment 1. Four 115 ± 12 kg (mean ± SD) Holstein bull calves were confined in individual calf metabolism stalls (Hansard, 1951). They were fed a mixed Trifolium pratense (IRN 1-01-405 [red clover]) and Dactylis glomerata (IRN 1-03-432 [orchard

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grass) hay ad libitum plus tap water and 10 g sodium chloride (NaCl) (IRN 6-04-153 [salt]) per day for 7 days prior to the experiment. After a 24-hr fast, two calves were given either 3% potassium chloride (135 mg K/kg body weight) or .9% NaCl solution via catheter into the left external jugular vein. Catheters were implanted (Hansard et al., 1951) 2 hr before infusion. The KCl or NaCl solutions were infused at the rate of 11 ml/min for 90 min by use of an intravenous injection set.

Blood was drawn twice from a catheter in the opposite external jugular vein prior to infusion and at 15, 30, 60, 90, 120, 150 and 210 min after initiation of infusion. One week later the procedure was repeated with the same calves, but either 2.35% NaCl or 1.14% (51 mg K/kg body weight) KCl was infused. Blood samples were heparinized and immediately placed in an ice bath. Sodium fluoride was added to separate samples intended for glucose determination. Plasma was separated by centrifugation and stored at -20°C in plastic tubes until analyzed. Plasma calcium (Ca), Mg and K were measured by atomic absorption spectrophotometry, glucose by enzymatic assay and immunoreactive insulin (IRI) by radioimmunoassay with a commercial kit modified by using a standard prepared from bovine insulin (24.3 IU/mg). Beef-pork glucagon did not cross-react with the antibody complex.

Statistical comparisons were made by analysis of variance (Federer, 1955) between preinfusion, infusion, and postinfusion periods.

Experiment 2. Four 161 ± 12 kg (mean ± SD) Holstein bull calves were randomly allotted to a semipurified ration (table 1) either adequate (.20% Mg) or deficient (.022% Mg) in magnesium. Confined in metabolism stalls, the calves were fed 3.6 kg of the ration per day and offered distilled water twice daily until blood Mg levels of un-supplemented calves fell below .7 mg/100 ml of plasma. All calves were then infused intravenously with 2% KCl (64 mg K/kg body weight) for 90 min as described in experiment 1. The 2% KCl was substituted for 3% KCl because 3% KCl was potentially too hazardous to the life of the calves. Blood samples, collected before infusions were begun and at 30, 60, 90 and 120 min after infusions were initiated, were treated, stored, and analyzed as described in experiment 1. The infusions were replicated 1 week later, and statistical comparisons were made between results obtained prior to, during, and after infusion.

Experiment 3. Eight Holstein cows weighing 660 ± 40 kg (mean ± SD) were each fed red clover-orchard grass hay (described in experiment 1) ad libitum and 1.3 kg of commercial 16% protein dairy concentrate daily. Each cow was fitted with a self-retaining corkscrew rumen trochar 5 days before intraruminal infusion with 550 g of KCl (440 mg K/kg body weight) dissolved in 3,000 ml distilled water.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>IRNa</th>
<th>Ration composition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn cobs</td>
<td>1-02-782</td>
<td>40.0</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>4-02-125</td>
<td>37.0</td>
</tr>
<tr>
<td>Starch</td>
<td>4-02-889</td>
<td>8.0</td>
</tr>
<tr>
<td>Soy proteinb</td>
<td>5-08-038</td>
<td>11.0</td>
</tr>
<tr>
<td>Vegetable oilc</td>
<td>4-05-077</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixtured</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>Vitamin D</td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>Vitamin A</td>
<td></td>
<td>1500</td>
</tr>
</tbody>
</table>

a International Reference Number.

b Assay protein C-1, Skidmore Enterprises, Cincinnati, OH.

c Hunt-Wesson Foods, Fullerton, CA 92634.
d Percentage composition of .07 cupric sulfate (IRN 6-01-718), .002 cobalt sulfate (6-01-561), 6.16 ferric citrate (6-01-858), 16.63 sodium chloride (6-04-153), 16.63 potassium phosphate (K2HPO4), 36.64 calcium carbonate (6-01-070), 16.63 calcium phosphate (6-01-083), .32 zinc chloride (6-05-552), .73 magnesium sulfate (6-03-051), .03 potassium iodide (6-03-760), and 6.16 magnesium oxide (6-02-757). Magnesium oxide was omitted from the magnesium-deficient ration.

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3 Cutter Laboratories, Berkeley, CA 94710.

4 Analytical methods for atomic absorption spectrophotometry, Perkin-Elmer Corporation, Norwalk, CT 06852.

5 Worthington Biochemical Corporation, Freehold, NJ 07728.


7 Sigma Chemical Co., St. Louis, MO 63178.

8 Security Mills, ConAgra, Inc., Knoxville, TN 37921.

9 Dr. Jorgensen’s Laboratories, Loveland, CO 80537.
Cows were confined in metabolism stalls (Hansard, 1951), and their left external jugular veins were catheterized 2 hr prior to infusion. The KCl solution was administered into the rumen at a constant rate over a 40-min period through 3-mm inside diameter plastic tubing inserted into the trochars. Blood was sampled and treated as described in experiment 1 before infusion and 30, 60, 90, 120 and 150 min after infusion was terminated.

Results were treated as a single-factor experiment with repeated measurements (Winer, 1971) and analyzed by analysis of variance employing regression with time as a continuous variable. Because of lack of homogeneity of variances, degrees of freedom were adjusted by the lower-bound technique of Greenhouse and Geisser (1959).

RESULTS

Experiment 1. Plasma K levels were elevated by intravenous infusion of both 3 and 1.14% KCl (P<.01) but not by either concentration of NaCl (P>.05) (figure 1). Plasma IRI increased (P<.01) during infusion of both KCl concentrations, but was not affected (P>.05) by equimolar solutions of NaCl (figure 2). Elevated IRI levels during infusion with 3% KCl were accompanied by (P<.05) decreases in plasma glucose. Plasma glucose did not change (P>.05) during infusion with 1.14% KCl or with either NaCl concentration (figure 2). Small but nonsignificant decreases in plasma Mg and Ca were observed in all calves (table 2).

Experiment 2. Intravenous infusion of 2% KCl elevated (P<.01) plasma K of both Mg-deficient and normal calves (figure 3). However, plasma K levels in Mg-deficient calves reached only 89% of the level in normal calves (P<.01). Plasma IRI concentrations of both Mg-deficient
TABLE 2. EFFECT OF INTRAVENOUS INFUSION OF KCI OR NaCI IN CALVES OR INTRARUMINAL INFUSION OF KCI IN COWS ON PLASMA MAGNESIUM AND CALCIUM CONCENTRATIONS

<table>
<thead>
<tr>
<th>Material infused, concentration and magnesium status of animal</th>
<th>Element and time in relation to infusion</th>
<th>mg/100 ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Magnesium</td>
<td>Calcium</td>
</tr>
<tr>
<td></td>
<td>Pre  During  Post</td>
<td>Pre  During  Post</td>
</tr>
</tbody>
</table>

Experiment 1 (calves)\(^a\)

| KCl, normal Mg | 1.14% | 1.82 ± 0.07 | 1.79 ± 0.05 | 1.85 ± 0.05 | 9.0 ± 0.2 | 9.0 ± 0.1 | 9.4 ± 0.2 |
| KCl, normal Mg | 3%    | 2.38 ± 0.02 | 2.29 ± 0.02 | 2.31 ± 0.03 | 9.5 ± 0.1 | 9.4 ± 0.1 | 9.4 ± 0.1 |
| NaCl, normal Mg| 0.9%  | 2.29 ± 0.06 | 2.27 ± 0.04 | 2.26 ± 0.03 | 9.6 ± 0.1 | 9.4 ± 0.1 | 9.5 ± 0.1 |
| NaCl, normal Mg| 2.35% | 2.00 ± 0.06 | 1.94 ± 0.05 | 1.88 ± 0.04 | 9.3 ± 0.1 | 9.2 ± 0.1 | 9.3 ± 0.1 |

Experiment 2 (calves)\(^b\)

| KCl | 2% | 2.20 ± 0.04 | 2.12 ± 0.02 | 2.13 ± 0.05 | 9.1 ± 0.1 | 8.8 ± 0.1 | 8.5 ± 0.1 |
| KCl | Mg deficient | 0.58 ± 0.04 | 0.58 ± 0.04 | 0.61 ± 0.08 | 9.4 ± 0.1 | 9.3 ± 0.1 | 9.2 ± 0.1 |

Experiment 3 (cows)\(^c\)

<table>
<thead>
<tr>
<th>Element</th>
<th>Time after infusion (min)</th>
<th>Pre 30 60 90 120 150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>1.76 ± 0.05</td>
<td>1.73 ± 0.09</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.8 ± 0.2</td>
<td>8.8 ± 0.2</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SE for four calves.
\(^b\)Mean ± SE for four calves each infused twice.
\(^c\)Mean ± SE for eight cows.

and normal calves also increased (P<.01) during KCl infusion but reached higher levels (P<.01) in Mg-deficient than in normal calves (figure 3). Plasma glucose levels decreased (P<.05) in normal but not Mg-deficient calves during infusion and were higher than preliminary measurements after infusion (P<0.05) in Mg-deficient calves (figure 3). Plasma Ca and Mg did not change (P>.05) during infusion in both groups (table 2).

Experiment 3. Regression curves for plasma K, IRI, and glucose of cows after rumen infusion with KCl are shown in figure 4. Statistical analysis revealed that increases in plasma IRI (P<.001) and K (P<.01) and decreases in plasma glucose (P<.001) were best represented, respectively, by third, third, and first order equations. These equations are:

\[
Y_{\text{IRI}} = 31.73 + .783X - .0117X^2 + .00004X^3 \quad (R^2 = .95); \\
Y_{\text{potassium}} = 17.95 + .209X - .0020X^2 + .00005X^3 \quad (R^2 = .96); \\
Y_{\text{glucose}} = 89.91 - .068X \quad (R^2 = .95); \]

where X = time in minutes after rumen KCl infusion was stopped and R^2 = coefficient of multiple determination. Neither plasma Ca nor Mg was affected (P>.05) by intraruminal KCl infusion (table 2).

Discussion

Elevated plasma K concentrations, whether resulting from intravenous (figures 1 and 3) or ruminal (figure 4) infusion, were accompanied by increased IRI levels in all experiments. Increased insulin was due to K since equimolar solutions of NaCl evoked no insulin response (figure 2). The plasma K-reducing action of insulin was recognized in nonruminants over 50 years ago (Briggs et al., 1923). Insulin may have an important physiological role in the regulation of serum K (Hiatt et al., 1972). Insulin infusion in insufficient concentrations to measurably affect glucose uptake in humans precipitates a net movement of K from extracellular to intracellular fluid (Zierler and Rabinowitz, 1964). Hiatt et al. (1974) have shown in dogs that insulin is indispensable for an adaptive
mechanism that protects the heart from rapid addition of excess K to the extracellular fluid.

Although plasma insulin increased in both Mg-deficient and normal calves when 2% KCl was infused intravenously, insulin levels were higher (P<.01) in deficient calves (figure 3). This could explain why plasma K did not attain levels as high in Mg-deficient as in normal calves during KCl infusion. Greater insulin response to K in Mg-deficient cattle could accelerate metabolic disorders of intermediary carbohydrate metabolism. Plasma glucose was decreased by KCl infusion, both intraruminally to cows (figure 4) and intravenously to normal calves (figures 2 and 3). Decreased plasma glucose may have been caused by inhibition of hepatic gluconeogenesis by elevated insulin (Hiatt et al., 1972).

Elevated plasma insulin (Field, 1964; Potter et al., 1974) and lowered glucose (Unger, 1973) are both potent stimulators of glucagon secretion in nonruminants. Glucagon secretion is also increased by stress in humans (Bloom et al., 1973b) and in calves (Bloom et al., 1973a). Glucagon, in turn, elevates plasma glucose and stimulates pancreatic insulin secretion in humans (Samols et al., 1966) and in calves (Madsen et al., unpublished data). Stimulation of glucagon release by abnormally high insulin may explain the increased plasma glucose observed in Mg-deficient calves after KCl infusion (figure 3).

Hypoglycemia and ketosis have been associated with the grass tetany syndrome (Wilcox and Hoff, 1974). Abnormal insulin secretion triggered by elevated plasma K (figures 2, 3 and 4) could be involved in the onset of these conditions. Glucagon could be involved by enhancing ketone production by increasing fat breakdown (Unger, 1973). Decreased energy intake often accompanying grass tetany (Grunes et al., 1970) can also lead to ketosis or even hypoglycemia by depriving the animal of certain precursors (especially propionic acid) for gluconeogenesis (Bergman, 1973). Ketosis, in turn, can further elevate insulin levels in cattle (Calhoun et al., 1962).

Prolonged elevation of insulin and thus of glucagon secretion in ruminants could eventually lead to depletion of gluconeogenic precursors and subsequent ketosis. Uncontrolled ketosis in turn could lower blood pH, triggering onset of grass tetany coma. The series of metabolic disturbances accompanying elevated insulin levels caused by abruptly increased K ingestion may well be found to play an important role in the etiology of grass tetany.

LITERATURE CITED
Figure 4. Changes in plasma potassium, insulin, and glucose of cows before potassium treatment (0 time) and at 30-min intervals after termination of intraruminal infusion with 550 g of KCl.


