SUBCLINICAL AMMONIA TOXICITY IN STEERS: EFFECTS ON BLOOD METABOLITE AND REGULATORY HORMONE CONCENTRATIONS

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ABSTRACT

The effects of subclinical NH₃ toxicity on circulating and regulatory hormone concentrations were investigated in seven Hereford steers. Ammonium chloride (NH₄Cl) was infused via a right jugular vein catheter at a rate of 12 µmol NH₄Cl·kg BW⁻¹·min⁻¹ for 240 min. This was preceded (PRE) and followed (POST) by saline infusions of 120 and 180 min, respectively. Blood samples were taken at 20-min intervals via a left jugular vein catheter. Metabolite and hormone concentrations during NH₄Cl and POST periods were compared to PRE values using the Student's t-test procedure. Plasma NH₃ was elevated rapidly (P < .001) and peaked at 503 µg/dl 220 min into NH₄Cl infusion. Plasma urea-N and glucose increased (P < .001) 39 and 12%, respectively, during NH₄Cl infusion and remained elevated 180 min POST. Whole blood L-lactate concentrations peaked (P < .05) at 18% above PRE levels between 160 and 240 min into the NH₄Cl infusion and gradually returned to PRE values, whereas pyruvate levels were not altered (P > .10). Plasma nonesterified fatty acids peaked (P < .001) at 94% above PRE levels 40 min into NH₄Cl infusion, thereafter declining to PRE concentrations. Whole blood acetoacetate and β-hydroxybutyrate concentrations were not altered (P > .10) by NH₄Cl administration. Plasma insulin concentration decreased (P < .05) 26 to 46% during NH₄Cl infusion and increased (P < .05) 89 to 122% during POST. Plasma glucagon levels were not altered by NH₄Cl infusion, so molar insulin:glucagon ratio changes resembled those of insulin. Plasma epinephrine, norepinephrine and dopamine did not vary (P > .10) with treatment. These results support the hypothesis that the hyperglycemia observed during hyperammonemia may result from an under-utilization of glucose by insulin-sensitive tissues.

(Key Words: Hyperammonemia, Ruminants, Glucose, Insulin, Glucagon, Catecholamines.)

Introduction

Ruminants can meet part of their dietary N requirement from nonprotein-N (NPN) from feed grade urea and biuret (Owens and Bergen, 1983). Cattle and sheep fed diets in which NPN makes up a large percentage of the total die-

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tary N have exhibited reduced growth, weight gain, N retention, feed efficiency and milk production (Chalupa et al., 1970; Chalupa, 1972). The decreased performance associated with NPN feeding may be linked to derangements in intermediary metabolism associated with elevated blood NH₃ concentrations (Prior et al., 1970; Chalupa, 1972; Spires and Clark, 1979; Emmanuel et al., 1982).

Hyperammonemia is associated with derangements in glucose metabolism in cattle (Spires and Clark, 1979; Symonds et al., 1981) and sheep (Prior et al., 1970; Edjtehadi et al., 1978; Barej and Harmeyer, 1979; Emmanuel et al., 1982). In addition, hyperammonemia affected plasma concentrations of insulin (Barej and Harmeyer, 1979; Emmanuel et al., 1982) and epinephrine (Emmanuel et al., 1982) in sheep, and glucagon concentrations in dogs (Strombeck et al., 1978).

There is little information documenting simultaneous changes in concentrations in blood of metabolites associated with energy metabolism and of regulatory hormones in hyperammonemic cattle. Circulating catecholamines, whose role in the etiology of NH₃ toxicity have been implicated repeatedly, have not been measured except for observations on a single sheep (Emmanuel et al., 1982). The objective of this experiment was to further characterize the effect of induced subclinical NH₃ toxicity on circulating carbohydrate, lipid and N metabolic intermediates and regulatory hormone concentrations in steers.

Materials and Methods

Animals and Diets. Seven Hereford steers (avg wt 295 kg) were housed indoors in individual tie stalls. The ambient temperature was maintained at 24 ± 4°C. A complete mixed diet (Table 1) was formulated to meet both protein and energy requirements necessary to sustain an ADG of .23 kg (NRC, 1984). To minimize postprandial changes in circulating metabolite and hormone concentrations, automatic feeders (Croom et al., 1982) were used to deliver equal portions of the daily ration at hourly intervals. Water was available ad libitum.

Experimental Procedure. Two hours prior to experimentation, catheters were inserted into the left and right jugular veins. A 4% sodium citrate-saline solution (w/v) was used as the anticoagulant. Experimental solutions were infused into the peripheral circulation via the right jugular catheter using a screw-driven syringe pump. Hyperammonemia was induced in each of the seven steers by continuously infusing an ammonium chloride (NH₄Cl)-saline solution (12 μmol NH₄Cl·kg BW⁻¹·min⁻¹; pH 7.2) at a rate of 1.23 ml/min for 240 min. This was preceded (PRE) and followed (POST) by .9% saline infusions of 120 and 180 min, respectively. At least 21 ml of blood was obtained via the left jugular catheter every 20 min. Immediately after sampling, the blood was centrifuged at 1,500 x g at 4°C for 10 min.

Analytical Methods. Plasma samples for metabolite and hormone analysis were stored at -20 or -70°C until further analysis. Whole blood L-lactate (LAC) and pyruvate (PYR) were determined enzymatically using lactate dehydrogenase (Sigma Tech. Bull No. 726 and 826, Sigma Chemical Co., St. Louis, MO). Whole blood acetoacetate (ACAC) and β-hydroxybutyrate (BOHB) were determined by using an enzymatic colorimetric assay (Heitmann et al., 1986). Plasma samples for ammonia-nitrogen (AMM) determination were stored at -70°C and assayed within 24 h (McCullough, 1967). Plasma urea-N (PUN) was analyzed by the assay of Chaney and Marbach (1962)

### TABLE 1. COMPOSITION AND CHEMICAL ANALYSIS OF EXPERIMENTAL DIET

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Cottonseed hulls</td>
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</tr>
<tr>
<td>Cracked corn</td>
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</tr>
<tr>
<td>Soybean meal</td>
<td>9.0</td>
</tr>
<tr>
<td>Cane molasses</td>
<td>7.0</td>
</tr>
<tr>
<td>Trace mineralized sal*</td>
<td>.5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>.3</td>
</tr>
<tr>
<td>Limestone</td>
<td>.2</td>
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</table>

<table>
<thead>
<tr>
<th>Chemical composition*</th>
<th>%</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>ADF</td>
<td>37.5</td>
</tr>
<tr>
<td>Ca</td>
<td>.36</td>
</tr>
<tr>
<td>P</td>
<td>.26</td>
</tr>
</tbody>
</table>

*Dry matter basis.

*Trace mineralized salt consisted of not less than .350% Zn, .200% Mn, .200% Fe, .030% Cu, .005% Co, .007% I and 96 to 98.5% NaCl.
and was corrected for endogenous AMM concentration. Plasma glucose was determined with an automated system utilizing membrane-immobilized glucose oxidase. Plasma nonesterified fatty acid (NEFA) concentration was determined by an enzymatic colorimetric technique.

Plasma insulin (INS) was assayed by the method of Koprowski and Tucker (1973). Guinea pig anti-bovine INS antisum was used at a 1/40,000 final dilution, whereas purified bovine INS (26.6 μU/ng) and bovine [125I]INS were used as standard and radioligand, respectively. Sheep anti-guinea pig antisum produced in our laboratory was used at a 1/4 dilution as the precipitating antibody. All samples were assayed in duplicate within a single batch. The intraassay cv as determined by pooled bovine plasma samples was 9.7%.

Immunoreactive glucagon (GLN) was assayed as described by Harris et al. (1979). The plasma was treated with benzamidine hydrochloride during preparation to reduce proteolytic degradation of GLN. Rabbit anti-porcine GLN antibody specific for small molecular weight (3,500 mol. wt) GLN was used at a final dilution of 1/225,000. Purified bovine GLN was the standard. Porcine [125I]GLN was the radioligand, and goat anti-rabbit immunoglobulin G was used at a 1/60 dilution as the precipitating antibody. All samples were assayed in duplicate and as a single batch. The intra assay cv as determined by pooled bovine plasma samples was 9.6%.

Blood for epinephrine (EPI), norepinephrine (NOR) and dopamine (DOP) analysis was treated with ascorbic acid (2 mg/ml), centrifuged, frozen using a dry ice-methanol bath and stored at -70°C until further preparation. Within 30 d, the catecholamines were isolated using activated alumina and 1 M HClO₄ and quantitated by using HPLC with electrochemical detection.

Statistical Analysis. Mean metabolite and hormone concentrations in NH₄Cl infusion and POST period samples for each sampling time were compared against a mean of six PRE samples using the Student's t-test procedure (Steel and Torrie, 1980) in the SAS package (SAS, 1982).

Results
Pre-infusion AMM concentrations ranged between 51 and 88 μg/dl. Infusion of NH₄Cl resulted in a greater than eightfold increase (P < .001) in AMM with plasma concentrations peaking at 503 μg/dl 220 min into the infusion (Figure 1a). Ammonia concentrations declined to PRE concentrations by 60 min POST. Average PRE PUN concentrations ranged from 8.1 to 8.5 mg/dl and increased gradually in response to NH₄Cl treatment (Figure 1b), peaking at 11.3 mg/dl 120 min POST. Plasma urea-N re-

![Figure 1](image-url)
mained elevated for the duration of the sampling period \((P < .001)\).

The effects of NH\(_4\)Cl infusion on circulating intermediates of carbohydrate metabolism are depicted in Figure 2. Pre-infusion plasma GLU averaged 75.8 mg/dl and tended to decrease \((P < .15)\) 20 min into NH\(_4\)Cl treatment (Figure 2a). Thereafter, GLU concentration increased steadily until it reached from 80.6 to 82.4 mg/dl during the last hour of NH\(_4\)Cl infusion \((P < .05)\). Glucose continued to rise until 80 min POST, when it peaked at 84.6 mg/dl \((P < .001)\) and decreased only slightly over the duration of the sampling period. Whole blood LAC exhibited a similar response to GLU, peaking at 18% above PRE levels during the last hour of NH\(_4\)Cl infusion \((P < .05); \text{Figure } 2b\). Lactate remained elevated 40 min POST \((P < .05)\) but decreased thereafter. No significant changes in blood PYR resulted from NH\(_4\)Cl infusion (Figure 2c).

Responses of plasma NEFA and whole blood ketone bodies to NH\(_4\)Cl treatment are depicted in Figure 3. Pre-infusion plasma NEFA ranged between 120 and 186 \(\mu\)Eq/liter and peaked \((P < .001); \text{Figure } 3a\) at 94% above PRE 40 min into NH\(_4\)Cl infusion. A steady decline in NEFA concentration followed, reaching PRE levels 140 min into NH\(_4\)Cl infusion and during the POST period. Whole blood ACAC and BOHB analyses were performed on samples obtained from three steers and did not appear to be altered by NH\(_4\)Cl treatment (Figures 3b and c).

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**Figure 2.** Effects of i.v. NH\(_4\)Cl administration (12 \(\mu\)mol NH\(_4\)Cl\(\cdot\)kg BW\(^{-1}\)\(\cdot\)min\(^{-1}\)) on plasma (A) glucose and whole blood (B) lactate and (C) pyruvate concentrations in steers. Each point represents the mean ± SE of seven animals. Ammonium chloride was infused for 240 min and was preceded (PRE) and followed (POST) by saline infusions of 120 and 180 min, respectively. Superscripts denote difference from PRE at \(^{aP} < .05\), \(^{bP} < .01\) and \(^{cP} < .001\).

**Figure 3.** Effects of i.v. NH\(_4\)Cl administration (12 \(\mu\)mol NH\(_4\)Cl\(\cdot\)kg BW\(^{-1}\)\(\cdot\)min\(^{-1}\)) on plasma (A) nonesterified fatty acid \((n = 7)\) and whole blood (B) acetoacetate \((n = 3)\) and (C) \(\beta\)-hydroxybutyrate \((n = 3)\) concentrations in steers. Each point represents the mean ± SE. Ammonium chloride was infused for 240 min and was preceded (PRE) and followed (POST) by saline infusions of 120 and 180 min, respectively. Superscripts denote difference from PRE at \(^{aP} < .05\), \(^{bP} < .01\) and \(^{cP} < .001\).
Mean ACAC concentrations remained low throughout the trial (3.1 to 9.2 µM), and BOHB averaged 254, 271 and 312 µM during PRE, NH₄Cl infusion and POST periods, respectively.

Plasma INS concentration averaged 29.4 to 36.7 µU/ml during PRE and was depressed (P < .05) by 26 to 46% during NH₄Cl infusion (Figure 4a). Cessation of NH₄Cl infusion caused a sustained increase (89 to 122%) in INS concentration. In contrast, plasma GLN levels were not altered by NH₄Cl treatment and averaged 187 pg/ml (Figure 4b). Mean molar INS:GLN ratios are depicted in Figure 4c. Molar INS:GLN ratios averaged 4.2 during PRE, decreased (P < .01) to 2.7 during NH₄Cl infusion and increased (P < .05) to 5.4 during POST, where it remained throughout the remainder of the experiment.

The responses of plasma EPI, NOR and DOP to NH₄Cl infusion are shown in Figure 5. No significant differences were noted in catecholamine levels as a result of NH₄Cl treatment. However, EPI was elevated (P < .10) 40 and 60 min POST (Figure 5a). Plasma catecholamines demonstrated great variability among steers and sampling times. Mean EPI and NOR values for PRE, NH₄Cl infusion and POST periods were 1,600 and 1,580; 1,818 and 1,542; and 2,094 and 1,632 pg/ml, respectively (Figures 5a and b). Dopamine values ranged from 196 to 308 pg/ml for all periods (Figure 3c).

Discussion

Intravenous infusion of NH₄Cl into the

Figure 4. Effects of i.v. NH₄Cl administration (12 µmol NH₄Cl/kg BW⁻¹*min⁻¹) on plasma (A) insulin and (B) glucagon concentrations and (C) molar insulin:glucagon ratios in steers. Each point represents the mean ± SE of seven animals. Ammonium chloride was infused for 230 min and was preceded (PRE) and followed (POST) by saline infusions of 120 and 180 min, respectively. Superscripts denote difference from PRE at aP < .05, bP < .01 and cP < .001.

Figure 5. Effects of i.v. NH₄Cl administration (12 µmol NH₄Cl/kg BW⁻¹*min⁻¹) on plasma (A) epinephrine, (B) norepinephrine and (C) dopamine concentrations in steers. Each point represents the mean ± SE of six animals. Ammonium chloride was infused for 240 min and was preceded (PRE) and followed (POST) by saline infusions of 120 and 180 min, respectively. Superscripts denote difference from PRE at aP < .10.
peripheral circulation elevated plasma AMM levels (503 µg/dl). Previous studies in which large dosages of urea were administered intraruminally to cattle considered peripheral blood AMM levels below 600 µg/dl as nontoxic (Bartley et al., 1976, 1981; Spies and Clark, 1979). Plasma AMM values during the PRE period are considerably lower than those reported previously (Spies and Clark, 1979; Emmanuel et al., 1982), which could be due to differences in sample preparation and handling procedures. In the present study, blood samples were centrifuged at 4°C immediately after sampling, deproteinized, quick-frozen and stored at -70°C. All samples were assayed within 24 h (McCullough, 1967). Our laboratory has found a 38% increase in AMM concentrations in plasma samples not deproteinized and stored at -20°C for 24 h (unpublished data). Furthermore, samples assayed 7 and 30 d after sampling and stored at -20°C had 50 and 54% greater AMM contents (unpublished data). Consequently, we think that our values represent true baseline measurements of circulating AMM concentrations in the peripheral blood of ruminants. The steers in this experiment exhibited neither reduced feed intake nor any of the neurological symptoms generally associated with acute ammonia toxicity (Bartley et al., 1976; Edjtehadi et al., 1978). Additionally, blood gas analyses performed on a representative number of samples were not indicative of metabolic acidosis. The average blood pH, pCO2 (mm Hg), pO2 (mm Hg) and HCO₃⁻ (mEq/liter) measurements during the PRE, NH₄Cl-infusion and POST periods were, respectively, 7.4, 44.5, 89.0 and 31.1; 7.4, 43.4, 111 and 26.7; and 7.4, 40.3, 110 and 26.0. Thus, the observed aberrations in circulating metabolite and hormone levels during NH₄Cl infusion were attributed to subclinical ammonia toxicity.

Plasma GLU concentrations exhibited a continuing 6 to 12% increase above PRE levels during NH₄Cl infusion (Figure 2a). Spires and Clark (1979) and Symonds et al. (1981) observed similar trends in cattle administered urea solutions directly into the rumen. Using radioisotope-dilution techniques to study whole-body GLU turnover in urea-dosed steers (Spires and Clark, 1979) and sheep (Emmanuel and Edjtehadi, 1981), previous workers speculated that the hyperglycemia characteristically associated with elevated AMM levels was due to decreased extrahepatic GLU utilization rather than to increased GLU production. To date, there have been no direct estimates of the effects of hyperammonemia in ruminants on hepatic and renal gluconeogenesis and GLU utilization by peripheral tissues in vivo. However, studies on the direct effects of AMM on glucose production by isolated hepatocytes demonstrated reduced gluconeogenesis from propionate (Weekes et al., 1978; Aiello et al., 1985) and PYR (Zahlten et al., 1974), whereas glucose production from LAC was stimulated (Zahlten et al., 1974; Aiello et al., 1985). In the present experiment, circulating levels of LAC, but not of PYR, were elevated during hyperammonemia (Figures 2b and c).

Plasma NEFA concentration increased rapidly during the 1st h of NH₄Cl infusion and then declined steadily to PRE levels (Figure 3a). Similar observations have been made in hyperammonemic sheep (Wiechetek et al., 1975; Garwacki et al., 1979). In addition, elevated blood ketone body concentrations have been associated with elevated AMM levels (Edjtehadi et al., 1978). However, in the present study, no significant changes in circulating ACAC and BOHB concentrations were noted in the limited number of samples assayed (Figures 3b and c). These changes in fatty acid metabolism may be related to reduced glucose supply and utilization by peripheral tissues. If glucose availability to muscle cells is reduced, NEFA and ketone bodies are oxidized at a greater rate (Madsen, 1983; Heitmann et al., 1986).

Infusion of NH₄Cl decreased plasma INS concentration as much as 46% but had no effect on GLN levels (Figure 4). Hypoinsulinemia in association with hyperammonemia has been reported in sheep (Barej and Harmeyer, 1979; Emmanuel et al., 1982). In rats, AMM does not appear to affect proinsulin biosynthesis by pancreatic ß-cells; however, decreases in reduced pyridine nucleotide concentrations have been observed during ß-cell intoxication with AMM, resulting in reduced INS secretion (Sener et al., 1978).

The molar INS:GLN ratio may be of greater physiological significance in regulating GLU metabolism than the circulating concentration of either hormone (Brockman, 1986). In the present study, the molar INS:GLN ratio was depressed during NH₄Cl infusion and then increased markedly during the POST period (Figure 4c). An elevated molar INS:GLN ratio promotes GLU utilization by peripheral tissues, whereas a reduced INS:GLN ratio stimulates gluconeogenesis and glycogenolysis, and hence
results in hyperglycemia (Brockman, 1986; Heitmann et al., 1986). The data presented in this paper do not conflict with the hypothesis that the hyperglycemia characteristically observed in hyperammonemic animals is the result of a reduced molar INS:GLN ratio and reduced GLU utilization by INS-sensitive extrahepatic tissues rather than by an increased gluconeogenesis.

Because of their effects on pancreatic hormone secretion and glucose and lipid metabolism, previous studies implicated catecholamines in the etiology of ammonia toxicity in sheep (Wiechetek et al., 1975; Barej and Harmeyer, 1979; Garwacki et al., 1979; Emmanuel et al., 1982). Epinephrine and NOR are known to increase lipolysis, glycogenolysis and gluconeogenesis, decrease INS secretion and increase GLN secretion (Bassett, 1970; Wiechetek et al., 1975; Garwacki et al., 1979). Emmanuel et al. (1982) measured plasma EPI in one sheep experiencing acute ammonia toxicity and reported a 27-fold increase in EPI concentration; however, such a response was not found in this study (Figure 5). Although individual catecholamines did not increase with subclinical ammonia toxicity, total catecholamines tended to increase during NH₄Cl infusion and during POST. This may account for the increased plasma glucose concentrations during NH₄Cl infusion.

To our knowledge, this is the first study to document simultaneous changes in concentrations of key blood energy metabolites, catecholamines and pancreatic hormones in subclinically AMM-intoxicated steers. This investigation demonstrated two significant facts concerning the effects of hyperammonemia in steers. First, aberrations in GLU metabolism and associated changes in regulatory hormone concentrations can occur at much lower blood AMM levels than previously thought. Secondly, although circulating catecholamines may increase as a secondary response to stress associated with severe or clinical hyperammonemia (Emmanuel et al., 1982), our data suggest that catecholamines play a minor role during the subclinical phase of the disorder. Our data suggest that the aberrations in GLU metabolism observed in AMM-intoxicated steers is due to complex alterations in the mechanisms associated with the regulation of synthesis and utilization of GLU by hepatic and extrahepatic tissues. The rise in blood GLU concomitant to a decrease in the molar INS:GLN ratio may indicate a decreased GLU utilization by INS-sensitive tissues, rather than increased GLU production. More precise experiments defining the effects of AMM on GLU and regulatory hormone flux across splanchnic and peripheral tissues are needed to fully understand the effects of hyperammonemia on intermediary metabolism, and, consequently, performance of NPN-fed ruminants.

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