SUBCLINICAL AMMONIA TOXICITY IN STEERS: EFFECTS ON HEPATIC AND PORTAL-DRAINED VISCERAL FLUX OF METABOLITES AND REGULATORY HORMONES

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ABSTRACT

Four calves (avg wt 161 kg) were surgically fitted with indwelling catheters in the femoral artery and femoral, portal, hepatic and mesenteric veins to study the effects of subclinical ammonia toxicity on portal-drained viscera (PDV) and hepatic (HEP) net flux of key metabolites and pancreatic hormones. Hyperammonemia was induced via administration of ammonium chloride (NH₄Cl; 12 μmol·kg BW⁻¹·min⁻¹) via the femoral vein catheter for 240 min; infusions were preceded (PRE) and followed (POST) by 60- and 180-min control periods, respectively. Blood samples were obtained from the arterial catheters, and portal and hepatic vein catheters. Net flux rates were calculated by multiplying venoarterial differences by blood flow. Arterial plasma ammonia N peaked (P < .01) at 327 μg/dl; hepatic ammonia extraction increased (P < .01) from 10 to 23% during NH₄Cl infusion. Arterial plasma glucose concentrations increased (P < .05) during NH₄Cl infusion (90.5 vs 82.6 mg/dl) concomitant with trends toward a reduction in net HEP glucose output. Portal-drained visceral release of insulin did not increase (P > .10) during NH₄Cl infusion despite the steady rise in circulating glucose concentration; however, cessation of NH₄Cl infusion resulted in a 109% increase (P < .05) in PDV insulin release at +60 min POST. Plasma L-lactate, nonesterified fatty acids, urea N and glucagon concentrations and net fluxes were variable throughout the experiment. Results tend to indicate that hyperammonemia reduced hepatic glucose output and glucose-mediated pancreatic insulin release.

(Key Words: Hyperammonemia, Ruminants, Glucose, Insulin, Splanchnic.)


Introduction

Ruminants are capable of meeting a portion of their dietary N requirement with nonprotein N (NPN) sources. However, cattle, sheep and goats fed diets containing a large percentage of NPN have exhibited suboptimal performance (Chalupa, 1972; Owens and Bergen, 1983). Elevated blood ammonia arising from the improper use of NPN has been associated with disturbances in intermediary metabolism that may reduce animal performance (Chalupa, 1972).

Hyperammonemia has been associated with derangements in glucose metabolism in cattle (Spires and Clark, 1979; Symonds et al., 1981; Fernandez et al., 1988) and sheep (Barej and
The effects of elevated blood ammonia on circulating levels of insulin (Barej and Harmeyer, 1979; Emmanuel et al., 1982; Fernandez et al., 1988) and glucagon (Fernandez et al., 1988) also have been documented in ruminants. To date, there has been no direct measurement of glucose net flux across the liver of hyperammonemic cattle. Furthermore, there are no studies that examine the effects of elevated blood ammonia on portal-drained visceral insulin and glucagon release. The objective of this experiment was to study the effects of induced subclinical ammonia toxicity on hepatic and portal-drained visceral net flux of glucose, key energy and N metabolic intermediates, and on insulin and glucagon.

Materials and Methods

Animals and Diet. Four Holstein steer calves (avg wt 161 kg) were housed indoors in individual tie stalls. A lighting schedule consisting of 11 h of light and 13 h of darkness was used to simulate natural photoperiod exposure; ambient temperature was maintained at 24 ± 4°C. A concentrate-based diet (Table 1) was formulated to meet both protein and energy requirements necessary to sustain an ADG of 1.7 kg (NRC, 1984). In order 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.

Surgical Procedure and Maintenance of Catheters. Chronic indwelling catheters were implanted into the portal, hepatic and mesenteric veins (Katz and Bergman, 1969b) and femoral artery and vein (Dougherty, 1981). Tygon catheter material9 was treated with TDMAC-Heparin complex10 and sterilized using ethylene oxide gas. Calves were fasted approximately 48 h and water was withheld 12 to 18 h prior to surgery. Surgeries under general anaesthesia, averaged 4 h in length. Catheters were introduced through the femoral artery and femoral vein and inserted approximately 30 cm so as to reside in the caudal aorta and posterior vena cava, respectively. Catheters were tested, filled with sterile heparinized saline and exteriorized. Catheters were checked one to three times daily and filled with heparinized saline (200 U/ml) except prior to and including experimental days, when a 4% sodium-citrate solution was used.

Experimental Protocol. Experiments were conducted 5 to 14 d following surgery when calves had 1) recovered fully from the surgery, 2) been on full feed for a minimum of 3 d, 3) had all catheters functioning and 4) had normal rectal temperatures (101.5°C). On experiment days, a 5% (wt/vol) para-aminohippuric acid-saline solution (PAH) was infused into the mesenteric vein (M) for 60 min prior to and during the trial. Blood samples were withdrawn simultaneously from the arterial catheters and the portal and hepatic vein catheters at 20-min intervals for 1 h during the predose (PRE). 9% saline infusion period. During the treatment period, hyperammonemia was induced by infusing an NH₄Cl-saline solution (12 μmol NH₄Cl·kg BW⁻¹·min⁻¹; pH 7.4) via the femoral vein catheter for 240 min. Blood samples were obtained at 15, 30, 60, 120, 180 and 240 min. A 180-min posidose (POST) .9% saline infusion period followed; blood samples were obtained at +15, +30, +60, +120 and +180 min following cessation of NH₄Cl infusion. Para-aminohippuric acid and NH₄Cl solutions were sterile and infused at .765 ml/min using a screw-driven syringe pump12. At each sampling time, 14 ml of blood were collected simultaneously from arterial and portal and hepatic vein catheters into tubes

TABLE 1. COMPOSITION OF EXPERIMENTAL DIET FED TO CALVES

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<thead>
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</tr>
<tr>
<td>Trace mineralized salt</td>
<td>.5</td>
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</tbody>
</table>

9 Norton Performance Plastics, Akron, OH.
10 Polysciences, Inc., Warrington, PA.
11 Aldrich Chemical Co., Milwaukee, WI.
12 Harvard Apparatus Co., Harvard, MA.
containing .07 ml 15% EDTA or 14 mg potassium oxalate and 17.5 mg sodium fluoride. Immediately after sampling, blood was allocated for PAH analysis, determination of packed cell volume (PCV) and the separation of plasma by centrifugation of blood at 1,200 x g for 10 min at 4°C. Plasma samples were stored at -20°C until further analyses. Plasma for ammonia N determination was quick-frozen using a methanol-dry ice bath and stored at -70°C until further analysis.

**Analytical Methods.** Whole blood flow (BF) rates were determined by the indicator-dilution method using PAH as described by Katz and Bergman (1969a). Plasma ammonia N was determined within 24 h of sampling and plasma urea N was analyzed using procedures described previously (Fernandez et al., 1988). Plasma urea N values were corrected for ammonia N concentrations. Plasma glucose and L-lactate were determined with a Model 27 YSI Industrial Analyzer using membrane immobilized glucose and lactate oxidase enzymes. Plasma nonesterified fatty acid concentrations were determined using an enzymatic colorimetric technique. Plasma insulin and glucagon were measured using techniques described previously (Fernandez et al., 1988). All samples were assayed in duplicate in a single radioimmunoassay run. Intra-assay CV averaged 9.3% and 9.4% for insulin and glucagon, respectively.

**Calculations.** Whole blood flow rates through the portal-drained viscera and liver were calculated using the Fick Principle:

\[
BF = \frac{P_{PAH}}{C_{v}^{PAH} - C_{a}^{PAH}}
\]

where BF is the whole blood flow rate through the tissue (liters/min), \(P_{PAH}\) is the infusion rate of PAH (o.d. units/min) and \(C_{v}^{PAH}\) and \(C_{a}^{PAH}\) are the PAH o.d. units (o.d. units/liter) in venous and arterial blood, respectively. Portal and hepatic vein flow rates were measured directly; hepatic artery flow was calculated from the differences between these two. Plasma flow (PF) rates were calculated as follows:

\[
PF = (1 - PCV) \times BF
\]

where PF is the plasma flow through the tissue (liters/min), PCV is the packed cell volume and BF is the whole blood flow rate (liters/min).

Metabolite and hormone net flux across the portal-drained viscera (PDV), liver (HEP) and total splanchnic (TSP) vascular beds were calculated using the following equations:

\[
PDV = P_{Fp} \times (C_{p} - C_{a})
\]

\[
HEP = P_{Fp} \times (C_{h} - C_{p}) + P_{Fa} \times (C_{h} - C_{a})
\]

\[
TSP = P_{Fh} \times (C_{h} - C_{a}) = PDV + HEP
\]

where \(P_{Fa}, P_{Fp}\) and \(P_{Fh}\) are plasma flow rates (liters/min) in the artery, portal and hepatic veins, and \(C_{a}, C_{p}\) and \(C_{h}\) are the metabolite or hormone concentrations in the arterial and portal and hepatic vein plasma samples. Positive net flux indicates a net metabolite release; negative net flux indicates a net uptake by the respective tissue.

Hepatic extraction ratios (HEXR) were calculated for each metabolite and hormone as follows:

\[
HEXR = \frac{HEP}{(P_{Fp} \times C_{p}) + (P_{Fa} \times C_{a})}
\]

where HEP is the hepatic net flux (conc. units/min), \(P_{Fa}\) and \(P_{Fp}\) are arterial and portal plasma flow rates (liters/min), and \(C_{a}\) and \(C_{p}\) are plasma metabolite or hormone concentrations in the artery and portal vein.

**Statistical Analysis.** Metabolite and hormone concentrations and net flux were analyzed using least squares ANOVA (Steel and Torrie, 1980). Each vessel or vascular bed was analyzed separately with calf and time as main effects. Moreover, NH₄Cl infusion and POST period sample means were compared by students t-test against a mean of four PRE samples. Similarly, concentrations and net fluxes were compared across vessels and vascular beds. Each sampling time was analyzed separately with calf and vessel or vascular bed as main effects. All statistical analyses were performed utilizing the SAS package (SAS, 1982). Sampling times with only one observation were not included in the analysis.

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13Becton Dickinson Vacutainer Systems, Rutherford, NJ.
14Yellow Springs Instruments Co., Yellow Springs, OH.
15Wako Pure Chemical Industries, Osaka, Japan.
Results

Portal vein, femoral artery and femoral vein catheters remained fully functional, whereas hepatic vein catheters proved difficult to maintain throughout the course of the experiment. Only one calf, number 45, completed the experiment with all catheters functional. Two other calves, 42 and 54, bled intermittently from the hepatic vein catheter; hepatic vein samples from the fourth calf, 44, were obtained during PRE only. Metabolite concentrations, hormone concentrations, flow rates and net flux rates are represented as sample means.

Whole blood and plasma flow rates for the portal vein, artery and hepatic vein are listed in Table 2. Blood flow measurements for the portal vein, artery and hepatic vein averaged 6.85, 2.12 and 8.83 liters/min, respectively, resulting in a mean portal:hepatic ratio of .776. Portal BF and PF were reduced (P < .05) at 120 min into the NH4Cl infusion and at +30 and +180 min POST compared with PRE baseline values. Hepatic artery and vein BF and PF were not altered by infusion. Mean arterial PCV values were 25.7, 24.4, 23.1 and 19.2% for calves 42, 44, 45 and 54, respectively.

The effects of NH4Cl infusion on mean plasma ammonia N levels, net fluxes and HEXR are listed in Table 3. Infusion of NH4Cl increased (P < .01) ammonia N concentrations to peak at values 240, 65 and 93% above PRE levels in arterial, portal and hepatic vein plasma samples, respectively. Hepatic vein ammonia N concentrations were not altered (P > .10) by NH4Cl infusion until 240 min into the infusion (P < .01). By comparison, arterial and portal ammonia N levels rose sharply within 15 min of NH4Cl infusion. Cessation of NH4Cl infusion resulted in a rapid decline in ammonia N concentrations in arterial and hepatic blood, whereas portal ammonia N concentration tended to remain elevated above PRE values throughout the POST period. Increased net HEP uptake and a tendency for reduced PDV release resulted in increased TSP clearance of ammonia N during NH4Cl infusion (Table 3). An enhanced PDV release of ammonia N was observed upon termination of NH4Cl infusion. Hepatic extraction ratio of

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PRE = control period prior to infusion; POST = postdosing control period.
12 μmol NH4Cl·kg BW−1·min−1.
Number of observations.
Blood flow rate.
Plasma flow rate.
Calculated value (H − P = A).
Different from PRE, P < .05.
**Different from PRE, P < .01.
ammonia N was enhanced ($P < .01$) 10 to 23% during NH$_4$Cl infusion (Table 3).

Ammonium chloride infusion tended to increase plasma urea N concentrations 19, 21 and 46% above PRE in samples from arterial and portal and hepatic vein catheters, respectively (Table 4). Plasma urea N remained elevated throughout the POST period. Net flux across the liver and PDV was variable (Table 4). Mean net HEP urea N output tended to increase during the 1st h of NH$_4$Cl infusion but decreased steadily thereafter. Total splanchnic net flux of urea N appears to have been diminished 180 min into the NH$_4$Cl treatment; however, the measured positive portal flux and negative hepatic flux are within error of measurement, indicating no true flux. Termination of NH$_4$Cl infusion resulted in 232 and 12% increases in net HEP and PDV output of urea N in calf 45 to yield a 117% increase in net TSP urea N release (data not shown).

The effects of NH$_4$Cl infusion on mean plasma glucose concentrations and net flux are shown in Table 5 and Figure 1. Ammonium chloride infusion increased ($P < .05$) mean glucose concentrations in arterial and portal blood by approximately 10% by 180 min (Table 5). There was a trend for elevated hepatic venous blood glucose concentration with NH$_4$Cl infusion. Mean PRE glucose net flux across the liver and PDV were 247.2 and -34.3 mg/min, respectively, resulting in a net TSP glucose release rate of 212.9 mg/min. Infusion of NH$_4$Cl tended to increase net glucose output by the liver to 482.8 mg/min at 60 min into the infusion (Table 5, Figure 1). Portal-drained viscera exhibited a sustained net uptake of glucose throughout NH$_4$Cl infusion, whereas net HEP glucose output decreased steadily after reaching peak levels at 60 min. Indeed, net glucose output by the liver decreased by as much as 510, 1,166 and 303 mg/min during NH$_4$Cl infusion in calves 42, 54 and 45, respectively (Figure 1). Total splanchnic output of glucose tended to decline later in the NH$_4$Cl infusion period (Table 5). Calf 45 exhibited a 2.6-fold increase in net TSP glucose output 15 min POST, but thereafter did not reach PRE net flux rates until +60 min POST (data not shown). Portal-drained viscera net clearance of glucose was increased between 48.6 and 77.5% during the 1st h of the POST period (Table 5).

Mean plasma lactate concentrations, net flux and HEXR are reported in Table 6. Infusion of NH$_4$Cl had a tendency to reduce lactate levels in arterial and portal blood and decreased ($P < .05$) hepatic venous blood lactate concentrations by 40% at 240 min into NH$_4$Cl infusion; concentrations returned to PRE values thereafter. The calves exhibited net PDV release (50.8 mg/min) and HEP uptake (-64.3 mg/min) of lactate during PRE, resulting in a net TSP uptake. Although not statistically different, infusion of NH$_4$Cl enhanced HEP clearance and reduced PDV release by 31 to 121% and 50%, respectively, yielding a net increase in lactate uptake by the TSP tissues. In calf 45, termination of NH$_4$Cl infusion resulted in increased PDV release and HEP uptake of lactate, with net TSP uptake increasing by 1.088% above PRE 120 min POST (data not shown). Hepatic extraction ratio of lactate was not altered ($P > .10$) by NH$_4$Cl infusion.

The effects of NH$_4$Cl infusion on plasma nonesterified fatty acid concentrations, net flux and HEXR are shown in Table 7. Predose nonesterified fatty acid concentrations were 141, 158 and 143 µEq/liter; concentrations peaked at 298, 326 and 392 µEq/liter 30 min into NH$_4$Cl infusion period in arterial and portal and hepatic venous blood, respectively. Plasma nonesterified fatty acid concentrations declined steadily throughout the remainder of the NH$_4$Cl infusion period and POST period. Net HEP flux of nonesterified fatty acid switched from net uptake (-132 mEq/min) to
<table>
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<th>POST&lt;sup&gt;b&lt;/sup&gt;, min</th>
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<td>490**</td>
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<sup>a</sup>Each mean represents four calves (n = 4) unless otherwise noted.
<sup>b</sup>PRE = control period prior to infusion; POST = posdosing control period.
<sup>c</sup>12 µmol NH<sub>4</sub>Cl·kg BW<sup>-1·min</sup><sup>-1</sup>.
<sup>d</sup>Hepatic net flux.
<sup>e</sup>Portal drained viscera; net flux.
<sup>f</sup>Total splanchnic net flux.
<sup>g</sup>Hepatic extraction ratio.
<sup>h</sup>,<sup>i</sup>,<sup>j</sup>Number of observations: <sup>h</sup>n = 3; <sup>i</sup>n = 2; <sup>j</sup>n = 1.
<sup>*</sup>Different from PRE (P < .05).
<sup>**</sup>Different from PRE (P < .01).
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<sup>a</sup>Each mean represents four calves (n = 4) unless otherwise noted.

<sup>b</sup>PRE = control period prior to infusion; POST = postdosing control period.

<sup>c</sup>12 μmol NH₄Cl·kg BW⁻¹·min⁻¹.

<sup>d</sup>Hepatic net flux.

<sup>e</sup>Portal-drained viscera net flux.

<sup>f</sup>Total splanchnic net flux.

<sup>g,h,i</sup>Number of observations: <sup>g</sup>n = 3; <sup>h</sup>n = 2; <sup>i</sup>n = 1.

*Different from PRE (P < .05).
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<td>112.9(^h)</td>
<td>85.4(^i)</td>
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<td>316.8(^i)</td>
<td>110.2(^b)</td>
<td>75.3</td>
<td></td>
</tr>
<tr>
<td>PDV(^e)</td>
<td>-34.3</td>
<td>-20.7</td>
<td>-32.1</td>
<td>-86.4</td>
<td>-77.7</td>
<td>-15.3</td>
<td>-55.1</td>
<td>-93.9</td>
<td>-97.8</td>
<td>-81.9</td>
<td>-52.2</td>
<td>-81.9</td>
<td>33.9</td>
<td></td>
</tr>
<tr>
<td>TSP(^f)</td>
<td>212.9(^h)</td>
<td>170.0(^h)</td>
<td>382.4(^h)</td>
<td>396.4(^h)</td>
<td>98.9(^h)</td>
<td>-132.0(^h)</td>
<td>57.8(^h)</td>
<td>-8.5</td>
<td>-130.2(^i)</td>
<td>142.0(^i)</td>
<td>264.6(^i)</td>
<td>28.3(^h)</td>
<td>74.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Each mean represents four calves (n = 4) unless otherwise noted.
\(^b\) PRE = control period prior to infusion; POST = postdosing control period.
\(^c\) 12 \(\mu\)mol NH\(_2\)Cl·kg BW\(^{-1}\)·min\(^{-1}\).
\(^d\) Hepatic net flux.
\(^e\) Portal-drained viscera net flux.
\(^f\) Total splanchnic net flux.
\(^\#\) Number of observations: \(n = 3\); \(^h\) \(n = 2\); \(^i\) \(n = 1\).

\(^*\) Different from PRE (P < .05).
TABLE 6. EFFECTS OF L.V. NH₄Cl ADMINISTRATION ON MEAN PLASMA LACTATE CONCENTRATIONS, NET FLUXES, AND HEPATIC EXTRACTION RATIO

<table>
<thead>
<tr>
<th>Item</th>
<th>PRE⁵, min</th>
<th>NH₄Cl infusion, min</th>
<th>POST⁶, min</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>00</td>
<td>15</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Arterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma concentration, mg/dl</td>
<td>4.62</td>
<td>4.25</td>
<td>4.58</td>
<td>4.08</td>
</tr>
<tr>
<td>Portal vein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>5.49</td>
<td>5.23</td>
<td>5.15</td>
<td>4.95</td>
</tr>
<tr>
<td>Net flux, mg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEP⁷</td>
<td>-64.3h</td>
<td>-84.5i</td>
<td>-85.0i</td>
<td>-141.9b</td>
</tr>
<tr>
<td>PDV⁸</td>
<td>50.8</td>
<td>51.2</td>
<td>29.0</td>
<td>50.3</td>
</tr>
<tr>
<td>TSP⁹</td>
<td>-13.5h</td>
<td>-33.3i</td>
<td>-56.0i</td>
<td>-91.6b</td>
</tr>
<tr>
<td>HEXR⁸</td>
<td>.19h</td>
<td>.29j</td>
<td>.32i</td>
<td>.32i</td>
</tr>
</tbody>
</table>

*Each mean represents four calves (n = 4) unless otherwise noted.

PRE = control period prior to infusion; POST = postdosing control period.

12 mmol NH₄Cl kg BW⁻¹ min⁻¹.

²Hepatic net flux.

²Portal-drained viscera net flux.

²Total splanchnic net flux.

²Hepatic extraction ratio.

⁵Number of observations: h = 3; i = 2; j = 1.

*Different from PRE (P < .05).
TABLE 7. EFFECTS OF I.V. NH₄Cl ADMINISTRATION ON MEAN PLASMA NONESTERIFIED FATTY ACID CONCENTRATIONS

<table>
<thead>
<tr>
<th>Item</th>
<th>PRE* min</th>
<th>NH₄Cl infusion min</th>
<th>POST* min</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>00</td>
<td>15</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Arterial</td>
<td>141</td>
<td>221</td>
<td>298</td>
<td>277</td>
</tr>
<tr>
<td>Portal vein</td>
<td>153</td>
<td>159</td>
<td>223</td>
<td>326</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>143h</td>
<td>327l</td>
<td>392j</td>
<td>289b</td>
</tr>
</tbody>
</table>

**Plasma concentration, μEq/liter**

<table>
<thead>
<tr>
<th>Item</th>
<th>Net flux, mEq/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPd</td>
<td>-132h</td>
</tr>
<tr>
<td>PDVc</td>
<td>103</td>
</tr>
<tr>
<td>TSFf</td>
<td>-28h</td>
</tr>
<tr>
<td>HEXRg</td>
<td>12h</td>
</tr>
</tbody>
</table>

**Notes:**
- Each mean represents four calves (n = 4) unless otherwise noted.
- PRE = control period prior to infusion; POST = postdosing control period.
- 12 μmol NH₄Cl/kg BW⁻¹min⁻¹.
- Hepatic net flux.
- Portal-drained viscera net flux.
- Total splanchnic net flux.
- Hepatic extraction ratio.

*b,i, Number of observations: b = 3, i = 2, h = 1.
* Different from PRE (P < .05).
net release (180 mEq/min), altering HEXR (P < .05) 15 min into NH₄Cl infusion.

Mean plasma insulin concentrations during PRE, NH₄Cl infusion and POST periods, respectively, were 14.6, 14.9 and 16.0 μU/ml for arterial blood, 17.6, 18.0 and 21.3 μU/ml for portal blood and 17.6, 18.2 and 13.6 μU/ml for hepatic venous blood (Table 8, Figure 2). Predose net PDV release and HEP uptake of insulin averaged 16.6 and -4.5 mU/min, respectively, resulting in a net output of insulin by TSP. During infusion of NH₄Cl, net HEP insulin flux changed from uptake to release during the 1st h, increasing net TSP release of the hormone. Cessation of NH₄Cl infusion resulted in a 109% increase (P < .05) in mean PDV insulin release and a concomitant increase in HEP uptake. Calf 45 exhibited net PDV release and HEP uptake of 74.9 and -51.6 mU/min, respectively, resulting in a net TSP insulin output of 23.3 mU/min at +60 min POST (Figure 2); this is a 79% increase in net TSP insulin release compared with PRE.

Ammonium chloride infusion did not affect (P > .10) plasma glucagon levels in arterial and portal blood, but there was a general tendency for decreased glucagon concentrations during NH₄Cl infusion and POST periods in hepatic venous blood (Table 9). Mean arterial, portal and hepatic blood glucagon concentrations throughout the entire experiment were 182.2, 192.8 and 165 pg/ml, respectively. Portal-drained visceral release of glucagon increased (P < .05) 312% above the PRE 240 min into NH₄Cl treatment.

Molar insulin:glucagon ratios are shown in Table 10. Mean PRE arterial and portal and hepatic venous blood insulin:glucagon molar ratios were 1.92, 2.14 and 2.15, respectively. Molar insulin:glucagon ratios were slightly elevated during NH₄Cl treatment. Although not significant (P > .10), cessation of NH₄Cl infusion tended to increase circulating insulin: glucagon molar ratios early in the POST period, which then was followed by a steady decline.

**Discussion**

Infusion of NH₄Cl at a rate of 12 μmol NH₄Cl·kg·BW⁻¹·min⁻¹ increased circulating ammonia N levels in all sampling sites, resulting in a peak arterial concentration of 327 μg/dl (Table 3). This is well below the concentration considered to be toxic in ruminants (Bartley et al., 1976; Spires and Clark, 1979; Bartley et al., 1981; Fernandez et al., 1988). Calves in this study did not exhibit any signs of acute ammonia toxicity and continued to consume their feed, which was delivered at hourly intervals. Furthermore, blood gas analysis conducted during a previous study (Fernandez et al., 1988) failed to detect metabolic acidosis due to the infusion of NH₄Cl. In calves 42 and 44, arterial pH, pCO₂, pO₂ and HCO₃ at 240 min into NH₄Cl infusion were 7.4, 42.1 mm Hg, 112 mm Hg and 26.2 mEq/liter, respectively, indicating that no acidosis had occurred. This suggests that changes in metabolite and hormone concentrations and fluxes can occur at ammonia levels previously thought to be nontoxic.

In the present study, BF was not affected consistently by NH₄Cl infusion. Barej et al., (1980) reported reduced hepatic BF rates in induced hyperammonemic sheep and suggested that α-adrenergic receptors were involved. Earlier work performed in our laboratory did not detect any changes in plasma catecholamine concentrations in similarly treated mature steers (Fernandez et al., 1988).

Some have attributed ammonia toxicity to the liver's inability to detoxify fully the increasing amounts of portal ammonia (Bartley et al., 1976; Symonds et al., 1981). In the present study, hepatic venous blood ammonia
### TABLE 8. EFFECTS OF I.V. NH₄Cl ADMINISTRATION ON MEAN PLASMA INSULIN CONCENTRATIONS, NET FLUXES AND HEPATIC EXTRACTION RATIO

<table>
<thead>
<tr>
<th>Item</th>
<th>PRE³, min</th>
<th>NH₄Cl infusion³, min</th>
<th>+15</th>
<th>POST³, min</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>00</td>
<td>15</td>
<td>30</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>Arterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal vein</td>
<td>17.6</td>
<td>17.3</td>
<td>16.4</td>
<td>13.5</td>
<td>22.8</td>
</tr>
<tr>
<td>PDV⁸</td>
<td>16.6</td>
<td>19.1</td>
<td>27.3</td>
<td>19.0</td>
<td>19.1</td>
</tr>
<tr>
<td>TSP⁹</td>
<td>12.1¹</td>
<td>24.6¹</td>
<td>45.9¹</td>
<td>36.6¹</td>
<td>-16.0¹</td>
</tr>
<tr>
<td>HEXR²</td>
<td>02²</td>
<td>-.11²</td>
<td>-.19²</td>
<td>-.08²</td>
<td>.16²</td>
</tr>
</tbody>
</table>

³Each mean represents four calves (n = 4) unless otherwise noted.
⁴PRE = control period prior to infusion; POST = postdosing control period.
⁵12 μmol NH₄Cl kg BW⁻¹min⁻¹.
⁶Hepatic net flux.
⁷Portal-drained viscera net flux.
⁸Total splanchnic net flux.
⁹Hepatic extraction ratio.

*Different from PRE (P < .05).
<table>
<thead>
<tr>
<th>Item</th>
<th>PRE&lt;sup&gt;b&lt;/sup&gt;, min</th>
<th>NH&lt;sub&gt;4&lt;/sub&gt;Cl infusion&lt;sup&gt;c&lt;/sup&gt;, min</th>
<th>POST&lt;sup&gt;b&lt;/sup&gt;, min</th>
<th>Pooled SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>00</td>
<td>15</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Arterial</td>
<td>185</td>
<td>174</td>
<td>180</td>
<td>170</td>
</tr>
<tr>
<td>Portal vein</td>
<td>193</td>
<td>177</td>
<td>194</td>
<td>191</td>
</tr>
<tr>
<td>Hepatic vein</td>
<td>189&lt;sup&gt;d&lt;/sup&gt;</td>
<td>171&lt;sup&gt;i&lt;/sup&gt;</td>
<td>168&lt;sup&gt;i&lt;/sup&gt;</td>
<td>148&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Plasma concentration, pg/ml</td>
<td>Net flux, ng/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEP&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-116.2&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-91.6&lt;sup&gt;i&lt;/sup&gt;</td>
<td>-149.9&lt;sup&gt;i&lt;/sup&gt;</td>
<td>-131.6&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>PDV&lt;sup&gt;e&lt;/sup&gt;</td>
<td>46.7</td>
<td>19.8</td>
<td>70.7</td>
<td>117.6</td>
</tr>
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<td>TSP&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-69.9&lt;sup&gt;g&lt;/sup&gt;</td>
<td>-71.3&lt;sup&gt;i&lt;/sup&gt;</td>
<td>-79.2&lt;sup&gt;i&lt;/sup&gt;</td>
<td>-14.0&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>HEXR&lt;sup&gt;g&lt;/sup&gt;</td>
<td>-.10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>.10&lt;sup&gt;i&lt;/sup&gt;</td>
<td>.15&lt;sup&gt;i&lt;/sup&gt;</td>
<td>.09&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Each mean represents four calves (n = 4) unless otherwise noted.

<sup>b</sup>PRE = control period prior to infusion; POST = postdosing control period.

<sup>c</sup>12 µmol NH₄Cl·kg BW⁻¹·min⁻¹.

<sup>d</sup>Hepatic net flux.

<sup>e</sup>Portal-drained viscera net flux.

<sup>f</sup>Total splanchnic net flux.

<sup>g</sup>Hepatic extraction ratio.

<sup>h,i,j</sup>Number of observations: h = 3; i = 2; j = 1.

*Different from PRE (P < .05).
TABLE 10. EFFECTS OF I.V. NH₄Cl ADMINISTRATION ON MEAN CIRCULATING INSULIN:GLUCAGON MOLAR RATIOS

| Item          | PREb min | NH₄Cl infusionc min | POSTb min | Pooled min NH₄I infusion | SEmin WST | min | -15 | +30 | +60 | +120 | +180 | +30 | +60 | +120 | +180 | Pooled | SE        |
|---------------|----------|---------------------|-----------|---------------------------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arterial      | 1.92     | 2.11                | 2.38      | 1.37                      | 2.73      | 2.02| 2.42| 2.80| 2.78| 2.63| 2.42| 1.05| 2.32| 1.72| 1.26| 0.26|
| Portal vein   | 2.14     | 2.58                | 1.86      | 1.70                      | 3.46      | 2.40| 2.71| 3.97| 3.53| 3.52| 1.52| 1.63| 1.42| 1.26| 0.26|
| Hepatic vein  | 2.15d    | 1.92e               | 3.21f     | 2.69d                      | 2.98d     | 4.05d| 2.37e| 3.16e| 2.32f| 1.93f| 1.15f| 1.72e| 1.26| 0.26|

*Each mean represents four calves (n = 4) unless otherwise noted.
bPRE = control period prior to infusion; POST = postdosing control period.
c12 μmol NH₄Cl/kg BW⁻¹·min⁻¹.
d,e,fNumber of observations: 4n = 3; 3n = 2; 2n = 1.
*Different from PRE (P < .05).

N concentrations remained relatively stable throughout much of the NH₄Cl infusion period, even though portal and arterial concentrations were markedly elevated (Table 3). Moreover, HEP net flux of ammonia N increased to -226.7 μg·kg BW⁻¹·min⁻¹ during NH₄Cl infusion from a PRE value of -79.5 μg·kg BW⁻¹·min⁻¹. Baird et al. (1975) reported a net HEP ammonia clearance rate of 452.1 μg·kg BW⁻¹·min⁻¹ in a lactating Holstein cow. It appears that the calves in this study would be capable of metabolizing greater amounts of ammonia N. Note that the liver cleared approximately 70% of the ammonia N presented to it; during hyperammonemia, the HEP increased to over 80% between 30 and 60 min of NH₄Cl infusion (Table 3), suggesting that increased uptake of ammonia during hyperammonemia was due not only to a concentration-dependent uptake of ammonia but also to physiological and biochemical mechanisms (Heitmann and Fernandez, 1986). Similar observations have been noted in sheep (Motyl et al., 1988; Orzechowski et al., 1988). Chalmers et al. (1971) and Bartley et al. (1981) speculated that elevated peripheral ammonia concentrations were the result of ruminal ammonia bypassing HEP circulation and detoxification rather than the inability of the liver to metabolize incoming ammonia. Our results support this theory.

In the present study, PRE net PDV uptake and HEP release of urea N averaged -80.7 and 205 μg·kg BW⁻¹·min⁻¹, respectively. Therefore, the PDV had a net uptake equivalent to almost 40% of HEP urea N production. Assuming that these baseline values are relatively constant in continuously fed animals, net PDV transfer of urea into the gut would be 18.7 g/d. Vercoe (1969) estimated that 17 to 20 net grams of endogenous urea were transported from the plasma to the rumen of cattle, whereas nonlactating (Huntington, 1982) and lactating dairy cows (Huntington, 1984) averaged 19 and 52 g daily, respectively. During NH₄Cl infusion, net PDV uptake of urea N decreased 79% concomitant to an increased portal blood urea N concentration and HEP urea N output (Table 4). This is perplexing in light of the fact that PDV uptake of plasma urea N and its subsequent transport into the gut is dependent on ruminal ammonia concentrations (Vercoe, 1969). Nevertheless, Huntington et al. (1983) reported a twofold increase in urea transfer from the PDV into the gut without observing differences in ruminal or plasma ammonia N in cows treated with intraruminal infusions of acetic acid.

Ammonium chloride-induced hyperammonemia affected circulating glucose concentrations and net fluxes (Table 5). Arterial (P < .05), portal (P < .05), and hepatic (NS) blood glucose levels were elevated as a result of NH₄Cl infusion. In calf 45, net HEP glucose output was markedly decreased during NH₄Cl infusion and did not return to PRE levels until 2 h POST. During PRE, net PDV glucose uptake averaged -213 μg·kg BW⁻¹·min⁻¹, which corresponds closely with values reported previously (Baird et al., 1975; Huntington, 1982). Portal-drained visceral net flux of glucose almost doubled during the POST period. Hyperglycemia is associated characteristically with elevated blood ammonia levels (Barej and Harmeyer, 1979; Garwacki et al., 1979; Spires and Clark, 1979; Emmanuel and
decreased glucose utilization by peripheral tissues rather than increased HEP production. In a recent study utilizing multicatheterized sheep, Orzechowski et al. (1988) observed a 50% decrease in HEP glucose output during infusion of NH₄Cl into the mesenteric vein (25 μmol·kg BW⁻¹·min⁻¹ for 6 h). In vitro studies on the effects of ammonia on glucose production and release by hepatocytes demonstrated reduced gluconeogenesis from propionate (Spires and Clark, 1975; Aiello et al., 1985), which could account for 27 to 55% of the glucose produced by ruminants (Bergman, 1983). In addition, elevated glucose can decrease HEP gluconeogenesis indirectly by increasing the insulin:glucagon molar ratio and, hence, decreasing the availability of key glucogenic metabolites to the liver (Bergman, 1983; Brockman and Laarveld, 1986). In this study, circulating insulin:glucagon molar ratios tended to increase during NH₄Cl infusion (Table 10). The initial increase in net HEP glucose output observed in the present study within the 1st h of NH₄Cl infusion may be due to increased glycolysis. Previous workers reported similar results both in vitro (Spires and Clark, 1975) and in vivo (Spires and Clark, 1979). However, in another study, hormones known to stimulate glycolysis were not affected in hyperammonemic steers (Fernandez et al., 1988).

Ammonium chloride infusion decreased hepatic venous blood lactate concentration concomitant with reduced PDV release and enhanced HEP uptake of lactate (Table 6). Between 20 and 40% of circulating lactate was extracted by the liver. Baird et al. (1975) reported similar PDV and HEP lactate net fluxes of 366 and 686 μg·kg BW⁻¹·min⁻¹, respectively, in dairy cows. Elevated circulating lactate levels have been observed in animals experiencing hyperammonemia (Garwacki et al., 1979; Emmanuel and Edjtehadi, 1981; Fernandez et al., 1988). Furthermore, gluconeogenesis from lactate was enhanced in isolated goat hepatocytes treated with NH₄Cl (Aiello et al., 1985).

In the present study, circulating concentrations of plasma nonesterified fatty acids tended to increase rapidly during the 1st h of NH₄Cl infusion, declining steadily thereafter (Table 7). Of particular interest was the net release (P < .05) of nonesterified fatty acids by the liver 15 min into NH₄Cl infusion. Normally, clearance of nonesterified fatty acids by the liver is concentration-dependent (Heitmann and Fernandez, 1986). In muscle cells, nonesterified fatty acids are oxidized at a greater rate when the availability of glucose is reduced (Madsen, 1983). Moreover, transport of glucose into muscle cells is insulin-sensitive, whereas nonesterified fatty acids enter the cell by passive diffusion (Madsen, 1983). Therefore, we suspect that the initial increase in nonesterified fatty acid concentration followed by the steady decline that is characteristic of induced hyperammonemia (Garwacki et al., 1979; Fernandez et al. 1988) is due to increased nonesterified fatty acid utilization by peripheral tissues.

To our knowledge, this is the first study to directly measure PDV release of insulin and glucagon in vivo during hyperammonemia. Infusion of NH₄Cl prevented a PDV insulin response to the hyperglycemia typically associated with elevated ammonia levels (Table 8). Additionally, elevated ammonia had a profound effect on HEP insulin clearance and HEXR during the 1st h of NH₄Cl infusion. In previous studies, sheep (Barej and Harmeyer, 1979; Emmanuel et al., 1982) and cattle (Fernandez et al., 1988) demonstrating hyperammonemia exhibited reduced peripheral insulin concentrations. The reasons for decreased insulin release are not clear. Beta-cell biosynthesis of proinsulin in vitro is not affected by ammonia (Sener et al., 1978); yet, a decrease in media concentration of insulin has been reported (Feldman and Lebovitz, 1971; Sener et al., 1978). Decreased β-cell reduced pyridine nucleotide concentrations have been observed in ammonia-intoxicated rat islets; that could inhibit insulin release (Sener et al., 1978). Further studies need to be conducted on the direct effects of ammonia on ruminant β-cell function. Cessation of NH₄Cl infusion resulted in a marked increase in net PDV release and HEP uptake of insulin. Circulating insulin concentrations have been shown previously to increase following experimentally induced hyperammonemia (Barej and Harmeyer, 1979; Fernandez et al., 1988), possibly as a result of increased PDV release such as
tended to occur in the present study (Figure 2).

Portal-drained visceral production of glucagon had a tendency to decrease between 60 and 180 min of treatment with NH₄Cl (Table 9). This response is similar to that observed in a previous study (Femandez et al., 1988). In dogs, i.v. infusion of ammonia solutions increased production of pancreatic and extrapancreatic glucagon (Strombeck et al., 1978). Glucagon concentrations in arterial and portal blood were not altered by NH₄Cl infusion; however, there was a tendency for hepatic venous blood glucagon levels to be reduced.

Intravenous infusion of NH₄Cl into steer calves resulted in elevated ammonia levels. Nonetheless, the apparent threshold of the liver to detoxify ammonia was not reached, so circulating ammonia remained at subtoxic concentrations. Hyperglycemia characteristically associated with hyperammonemia was noted; however, net glucose output by the liver was reduced. Pancreatic insulin release tended to be inhibited by ammonia; this was confirmed by trends toward elevated PDV insulin release and circulating molar insulin:glucagon ratios during POST. Hepatic binding of insulin may have been altered by ammonia (Fausel and Mulloy, 1983), whereas glucagon flux across TSP vascular beds was variable. Presumably, ammonia decreases glucose output by the liver and insulin release by the pancreas, which results in a hyperglycemic state primarily due to impaired glucose uptake by insulin-sensitive tissues. This may partially explain why animal performance is suboptimal with the feeding of certain diets containing NPN or high concentrations of dietary protein.

Implications

Suboptimal animal performance associated with increased incorporation of NPN in ruminant diets may be due to derangements in glucose metabolism. Even though plasma ammonia levels were below those previously considered to be toxic, hepatic glucose output and pancreatic insulin secretion were changed in calves, suggesting the occurrence of subclinical ammonia toxicity. Considering the absolute requirement of glucose for growth, fetal development and milk production, and the fact that ruminants rely almost entirely on hepatic gluconeogenesis to meet their glucose needs, decreased glucose output by the liver during hyperammonemia may explain the suboptimal performance exhibited by cattle, sheep and goats consuming diets containing large amounts of NPN.

Literature Cited


