Feed Intake of Goats and Sheep Following Acetate or Propionate Injections into Rumen, Ruminal Pouches, and Abomasum as Affected by Local Anesthetics

F. HEINRICH MARTIN1 and CLIFTON A. BAILE1
Department of Nutrition, Harvard School of Public Health
Boston, Massachusetts 02115

Abstract

Previous experiments indicated that changes in rumen fluid concentration of volatile fatty acids especially in the dorsal rumen may play a role in the control of feed intake of ruminants. Experiments were designed to test for influence of possible ruminal nerve receptors in this feeding response. In Experiment 1, water (control), M acetate, M propionate (pH 6.5), or M acetate or propionate plus 400 mg/liter of Carbocaine, Xylocaine, or Oxethazaine (local anesthetics), were injected into the dorsal rumen of goats during spontaneous meals. Carbocaine and M acetate depressed feeding less than acetate alone (P<.01). Oxethazaine was ineffective in preventing feed intake decrease caused by acetate (P<.01). Propionate alone decreased feed intake 14% versus controls, but combined with either Carbocaine or Xylocaine feeding decreased further. In Experiment 2, catheters were implanted on the right ruminal nerve. Xylocaine (2%) injected on this nerve tended to increase feeding slightly. Xylocaine combined with intraruminal M acetate injections tended to decrease feeding less than acetate alone. Xylocaine injections combined with intraruminal propionate tended to decrease feeding less than propionate alone. In Experiment 3, Pavlov type pouches with neural and blood supply intact were formed in an area innervated by the left ruminal nerve in goats and sheep. Both rumen and pouch were provided with cannulas. Animals were fed twice daily for 2 hr. Thirty ml M acetate, propionate, acetate plus Carbocaine (400 mg/liter), propionate plus Carbocaine, or rumen fluid for a control were injected into pouches on test days just prior to both feedings. Each type of pouch injection was tested for 5 consecutive days. After the second daily feeding, pouches were filled with fresh rumen fluid and left overnight. Feed intake as a per cent of control was decreased with acetate (1%) whereas acetate plus Carbocaine increased feeding by 11%. Propionate tended to decrease intakes more (14.1%) than propionate plus Carbocaine (1%). In Experiment 4 water (control), M acetate or M acetate plus Carbocaine (400 mg/liter) were injected through catheters into the abomasum of goats. Acetate alone decreased feeding (P<.02) more than acetate plus Carbocaine although more acetate was injected during the latter treatment. Results lend further support to the hypothesis of acetate receptors in the ruminal wall of goats and sheep.

Introduction

Acetate and propionate have been suggested for possible roles in the feedback system for hunger-satiety in ruminants. Intraruminal injections of acetate into the dorsal sac of sheep (8) and goats (5, 6) caused a decrease in feed intake. Increased acetate concentration in the rumen fluid is more effective in reducing feed intake than increase of acetate content with unchanged concentration (8). Feed intake decrease is proportional to the mmoles injected per meal if the digesta dilution is minimal. The feed intake decreases with acetate or propionate are much more than just compensation for the metabolizable energy of the salts injected into the rumen (6). Receptors were hypothesized to be located on the lumen side of the gastric areas in goats (4, 5) since feed intake is decreased much more by intraruminal than by intravenous injections of sodium acetate. The decreased feeding with acetate injections into the abomasum were probably due more to unphysiological levels of the metabolite than to acetate receptors in that area (9). Receptors for propionate may be the same as or similar
NEURAL RUMINAL RECEPTORS

Methods

General procedures. Adult female goats or sheep adapted to laboratory conditions and fed for at least 2 weeks were prepared surgically after a 24-hr fast, with Silastic® catheters either on the right ruminal nerve or intra-abomasally, and with rumen pouches or rumen fistulas or both. After recuperating for at least 10 days, the animals were started on experiments provided they had returned to their previous feeding level. All animals except those with rumen pouches were fed ad libitum. During experiments, a 2-day control preceded a 2-day treatment which was followed by a 2-day recovery period. All animals were individually caged in a room at 24 ± 1°C and fed a concentrates grain ration (Omolene®, Ralston Purina). Orts and water intakes were determined daily. Those animals which had been provided with rumen pouches were adapted to a daily schedule of two 2-hr feedings. All animals except those with rumen pouches were fed ad libitum. During experiments, a 2-day control preceded a 2-day treatment which was followed by a 2-day recovery period. All animals were individually caged in a room at 24 ± 1°C and fed a concentrate grain ration (Omolene®, Ralston Purina). Orts and water intakes were determined daily. Those animals which had been provided with rumen pouches were adapted to a daily schedule of two 2-hr feedings. Orts were measured after each meal while water intakes were determined only once daily. To test for differences in feed and water intakes resulting from test injections, paired t-tests were used.

Experiment 1. Intraruminal injections of acetate or propionate and local anesthetic. Goats were prepared with ruminal fistulas in the dorsal rumen and fitted with cannulas. Local anesthetic and 1.0 M sodium acetate or propionate solution (pH 6.5) were injected (5 ml/min) during spontaneous meals. A goat when eating broke a light beam impinging on a photocell which activated a peristaltic pump and a solenoid valve as described previously (5). A different local anesthetic was injected in each of three tests.

In the first test, three goats were injected during test days with sodium acetate, sodium propionate, 0.04% Carbocaine (Winthrop Laboratories, New York) or 0.04% Xylocaine (Astra Pharmaceutical Prod., Mass.), or 0.04% Xylocaine combined with either sodium acetate or propionate. In a third test, five goats were injected with sodium acetate, 0.04% Oxethazaine (Wyeth Laboratories, Philadelphia) and 0.04% Oxethazaine and sodium acetate. When sodium acetate was combined with Oxethazaine, the latter precipitated out of solution and, therefore, the solutions were injected simultaneously but separately. All tests were replicated.

Experiment 2. Local anesthetic injections on ruminal nerve. Three goats were prepared with Silastic catheters (.63 mm id by 1.0 mm od, Pilling Co., New York) implanted in the nerve sheath of the right ruminal nerve about half-way between the anterior and caudal portions of the dorsal sac and secured with 4-0 chromic gut. These catheters were then brought subcutaneously to the left paralumbar fossa near a cannula in the dorsal rumen. The catheters were connected to a syringe pump which was activated for about 15 sec at the initiation of each spontaneous meal; injections of metabolites into the rumen were throughout the duration of each meal. Injections of local anesthetic occurred no more than once an hour because the pump was connected to an automatic-reset 1-hr delay timer.

In one test, three goats were injected with 1.0 M sodium acetate, 2% Xylocaine (.5 ml/injection) or 2% Xylocaine (.5 ml/injection) and 1.0 M sodium acetate (5 ml/min). One goat died from a catheter infection and the experiment was not replicated. In a second test, the two surviving goats of the previous test were also injected with either 1.0 M sodium propionate (5 ml/min) or 2% Xylocaine (.5 ml/injection) and 1.0 M sodium propionate (5 ml/min). This test was replicated.

Experiment 3. Injections into ruminal pouches. Pavlov type pouches were surgically formed in an area innervated by the left ruminal nerve (dorsal blind sac) in three goats (45 kg) and three sheep (35 kg) adapting procedures described by Tsuda (15) and Perry (13) leaving intact the neural and blood supply. Both rumen and pouch were provided with permanent hard rubber cannulas to allow sampling and injections. Post-operative care consisted in washing out the pouch once daily with normal saline, and leaving 10 ml of saline and 100,000 IU of penicillin in it until bleeding subsided. Thereafter fresh rumen fluid was introduced daily in the pouches.

The solutions tested were 1.0 M sodium salts (all pH 6.5) of: a) acetate; b) propionate;
c) acetate plus Carbocaine (400 mg/liter); d) propionate plus Carbocaine, and e) rumen fluid which served as a control. On test days (5 consecutive days for each metabolite and rumen fluid) each pouch was injected with 30 ml of a test solution prior to feeding. After each 2 hr feeding, solutions were recovered quantitatively from the pouches which were rinsed and left filled with water between daily feedings. After the second daily feeding, pouches were filled with fresh rumen fluid and left overnight. Treatments were assigned at random to each animal, and each experimental period was followed by two recovery days during which the pouches were filled once daily with fresh rumen fluid. Once weekly, the recovered test solutions and rumen fluid were sampled for assay of short-chain fatty acid by gas chromatography (10).

Ruminal motility was monitored by a balloon filled with air in the rumen and pouch motility was measured by pressure changes of the pouch filled with the test solution. Rumen and pouch cannulas were connected by tubing to pressure transducers (Statham Instruments, Inc., Model 23Db) to measure frequency of contractions, and a photographic record was made with a polygraph (Electronics For Medicine, Model DR8). The pouches were tested for capillary fistulas by following by X-ray the distribution of a radiopaque substance injected into the pouch. In another test 30 ml of 1.0 M sodium acetate plus 14C polyethylene glycol (PEG, New England Nuclear Corp.) containing approximately 3 µCi/ml was injected into the pouches. After 2 hr the solution was recovered, quantitatively, and samples were prepared for radioisotope counting in a scintillation system and for gas chromatography, to determine percent recovery of labeled PEG and disappearance of sodium acetate.

**Experiment 4. Intra-abomasal injections of acetate and local anesthetic.** For this experiment, three goats were surgically prepared with Silastic catheters (1.25 mm id) in the lesser curvature of the abomasum. The end of each catheter had a 1 cm collar (5.0 mm od) implanted through the wall according to the method of Witzel (12). Each catheter was then brought subcutaneously into the right paralumbar fossa. Catheters were clamped closed and were flushed with water every day. The goats were injected with 1.0 M sodium acetate (5 ml/min), .04% Carbocaine (5 ml/min) or .04% Carbocaine combined with 1.0 M sodium acetate (5 ml/min). All tests were replicated.

**Results**

**Experiment 1. Intraruminal injections of acetate or propionate and a local anesthetic.** Feed intake data of goats during the different intraruminal injections of sodium salts of volatile fatty acids (acetate and propionate) and local anesthetics, either alone or in combination, are in Figure 1. Although neither local anesthetic affected feeding of the goats, acetate decreased feeding in these tests an average of 21% (P<.01) when compared with control days. Of the local anesthetics, only Carbocaine (226 mg/day) was effective (P<.08) in eliminating at least partially the feeding response (15.7% versus 21.1%) caused by acetate (530 mM/day) injected during spontaneous meals. Propionate alone (774 mM/day) tended to decrease feeding (14%, P>.3) when compared to control days, but propionate (640 mM/day) combined with Carbocaine (260 mg/day) or propionate (640 mM/day) combined with Xylocaine (256 mg/day) resulted in more severe depression of feed intakes (20%, P<.05 and 35%, P<.01).

**Experiment 2. Local anesthetic injections on ruminal nerve.** Feed intake was little affected when a local anesthetic (Xylocaine, 178 mg/day) was injected near the ruminal nerves of goats.
the dorsal sac area of goats at the initiation of spontaneous meals (8 to 10% greater, P>0.2), Figure 2. Xylocaaine injections (156 mg/day) combined with sodium acetate (461 mM/day) injected intraruminally decreased feeding 16.7% (P<0.1) while acetate injections alone (425 mM/day) decreased feeding 24% (P<0.01). The combined treatment of propionate (356 mM/day) and Xylocaaine (144 mg/day) decreased feeding 18.5% (P>0.3) while propionate alone (409 mM/day) decreased feeding 30% (P<0.01).

**Experiment 3. Injections into ruminal pouches.** Morning and total daily feed intakes and water intakes are in Figure 3. There was no difference in feeding responses between species. Relative changes of feed intake after acetate and propionate injections as well as the combination of metabolites plus Carbocaine were not significant. However, feed intakes expressed as a per cent of intake when rumen fluid was injected into the pouches (per cent of control) were decreased by acetate (7.2%) whereas the combination of acetate and Carbocaine increased feed intake 7% during the morning meal (14% difference between 2 treatments, P<0.01) (Fig. 4). A similar trend was also observed when the total daily intake (morning plus afternoon intakes) was expressed as a per cent of control; acetate decreased feeding 1% while acetate plus Carbocaine increased feed intake 11% (P<0.05). Propionate and propionate plus Carbocaine decreased feeding (14.5% and 1%). The difference between these two treatments was not significant (P>0.1). Water intakes were not significantly influenced by the ruminal pouch.
injections. Ruminal and pouch motility rates were variable and there were no significant treatment effects. From photographic tracings during tests it was observed at times that pouch and rumen contractions occurred simultaneously and at other times pouch contractions preceded rumen contractions. In no instance did Carbocaine eliminate pouch motility.

Table 1 presents volatile fatty acid absorption from the pouches of the goats and sheep during the different experimental periods. About 87% of the $^{14}$C PEG injected into the pouches was recovered after 2 hr; this indicates that there were few if any capillary fistulas since some loss from the cannulas was unavoidable during recovery. No capillary fistulas were detected by radiopaque substance injected into the pouch.

Table 1. Absorption of volatile fatty acid salts and rumen fluid from rumen pouches in goats and sheep.

<table>
<thead>
<tr>
<th></th>
<th>Absorbed after 2 hr</th>
<th>Absorbed after 2 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetic (mmoles)</td>
<td>Propionic (%)</td>
</tr>
<tr>
<td>Goats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 M NaAc</td>
<td>14.40</td>
<td>.....</td>
</tr>
<tr>
<td>1.0 M NaAc+C</td>
<td>14.30</td>
<td>.....</td>
</tr>
<tr>
<td>1.0 M NaProp</td>
<td>..... 14.0</td>
<td>.....</td>
</tr>
<tr>
<td>1.0 M NaProp+C</td>
<td>..... 9.4</td>
<td>.....</td>
</tr>
<tr>
<td>Rumen fluid</td>
<td>17.30</td>
<td>7.8</td>
</tr>
<tr>
<td>Sheep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 M NaAc</td>
<td>9.33</td>
<td>.....</td>
</tr>
<tr>
<td>1.0 M NaAc+C</td>
<td>7.33</td>
<td>.....</td>
</tr>
<tr>
<td>1.0 M NaProp</td>
<td>..... 12.70</td>
<td>.....</td>
</tr>
<tr>
<td>1.0 M NaProp+C</td>
<td>..... 13.33</td>
<td>.....</td>
</tr>
<tr>
<td>Rumen fluid</td>
<td>5.86</td>
<td>5.23</td>
</tr>
</tbody>
</table>

Journal of Dairy Science Vol. 55, No. 5
Experiment 4. Intra-abomasal injections of acetate and local anesthetic. Feed intake depression (38.8%, P<.02) with 694 mg/day of acetate injections into the abomasum (Fig. 5) is similar to that in another experiment (9). Injections of Carbocaine (393 mg/day) alone did not affect feeding (4.8% decrease, P>.6) while combined treatment of acetate (744 mg/day) plus Carbocaine (297 mg/day) resulted in a trend of a decreased feeding (26.8%, P>.2) although about 50 mg more acetate were injected during the combined tests than during acetate injection tests.

Discussion

From Experiments 1, 3 and 4, it appears that Carbocaine injected into the rumen, rumen pouches, and possibly the abomasum, suppressed neural signals due to changes in acetate concentration in these areas; however, no electrophysiological measurements have been made to confirm this. Carbocaine injected intraruminally during a spontaneous meal at a concentration of .04% does not affect rumen or pouch motility as determined by motility recordings. A goat eating a spontaneous meal of 10 min duration received 50 ml of .04% Carbocaine solution (20 mg Carbocaine/meal). Since intakes were near normal with the combined treatment (acetate plus Carbocaine) while lower with acetate alone, it appears that the injected dose is adequate to prevent those changes in feeding pattern (without affecting normal motility) which occur with acetate alone. The length and number of meals tend to decrease when feed intake is depressed by intraruminal acetate alone (6).

The other two local anesthetics injected into the rumen, Xylocaine and Oxethazaine, were not effective in eliminating decrease in feed intake caused by acetate or propionate. This lack of effect may be the result of differences in their chemical stability in the rumen, differences in ruminal epithelial sensitivity, etc. The action of Carbocaine is more rapid in onset and somewhat more prolonged than that of Xylocaine (11).

Receptors in the depression of feed intake caused by propionate may be in the ruminal veins in addition to being in the ruminal wall (3, 9). Small amounts of propionate injected into ruminal veins of sheep or goats during meals reduce feed intake much more than injections into mesenteric, portal, or jugular veins, carotid artery or ruminoreticularum. In Experiment 1, neither Carbocaine nor Xylocaine eliminated the effect of propionate injections; however, Carbocaine appeared to eliminate at least partially the effect of propionate injected into the pouches (Experiment 3) since feeding increased. This could be due to a more direct effect of Carbocaine on the left ruminal vein which drained the ruminal pouches. In Experiment 2, Xylocaine injected in the dorsal sac on the right ruminal nerve at the initiation of a spontaneous meal increased feed intake slightly and also partially eliminated the effect on feed intake of sodium acetate or propionate injected into the rumen. Although the difference between acetate and acetate and Xylocaine injections was not significant, during the latter, feeding decreased less (about 8%). A similar trend was observed when propionate was compared with propionate and Xylocaine.
Propionate alone decreased feeding 30% while the combined treatment decreased intakes only 18.5%. The response to Xylocaaine injections (increased feeding) was variable.

The variations can be attributed to several factors such as catheter placement on the nerve, area anesthetized during injections, rejection of catheter together with tissue proliferation at the site of implant, and possibly a decrease in permeability of the area to the local anesthetic. Since meal length and frequency as well as ruminal motility were not affected, it appeared that the goats ate a more normal meal when the combined treatment was given (acetate and Xylocaaine) than when acetate was given alone.

In Experiment 3, 30 mmole injections of acetate or propionate salts into ruminal pouches decreased feed intake when compared to the combination of either metabolite plus Carbocaine. Previous experiments (6, 7, 9) have shown that injections of about 76 mmoles of acetate per meal into the dorsal rumen of goats caused a concentration change of 15 to 20 mmoles at the end of a meal and significantly depressed feeding. When Carbocaine was combined with the volatile fatty acid salts in the present study, it appeared that neural signals by the metabolites, which decrease feeding, were suppressed at the ruminal wall. The concentration change caused in the pouches was much greater than that in the rumen in other experiments (approximately 570 mmolar at the end of the 2-hr feeding in the present study). Feed intake by goats and sheep tended to be less following metabolite injections into the pouches than following the combined treatment of acetate or propionate and Carbocaine which lends supporting evidence to the hypothesis of receptors for volatile fatty acids on the lumen side of gastric areas (e.g. dorsal sac of the pouches) than following the combined treatment days when compared to the response to acetate injected alone. When a local anesthetic (Xylocaaine) is injected near a ruminal nerve and acetate is simultaneously injected into the rumen, feeding tends to be decreased less than when acetate is injected alone. When acetate and Carbocaine are injected together into rumen pouches of goats or sheep during scheduled feedings, feed intakes are greater than when acetate is injected alone. This indicates that a neural signal instead of a humoral factor is responsible for a decrease in feeding when acetate concentration is increased in the rumen.

The data of Experiment 4 show that acetate injected into the abomasum decreased feed intake markedly compared to control days. These injections caused a substantial increase in acetate concentration in abomasal fluid. The average injection per meal is about 70 mmoles for this treatment. Goats normally eat for about 15 min per meal (4, 7, 8), and the approximate rate of injection was 4.5 mmoles per min. The amount injected per meal was about one-half that injected by Ash (1), who showed that acetate injections into the abomasum can cause greater amplitude and frequency of reticular contractions which could disturb the animal and possibly slow the rate of eating. That acetate may be actively absorbed from the small intestine (14) soon after it is injected into the abomasum probably does not help explain the feed intake depression since high concentrations of acetate in the blood are not effective in decreasing feed intake of goats (5). Of interest is the result by the combined injection of acetate and Carbocaine, since a higher level of acetate was injected during this treatment than when acetate was injected alone (744 versus 694 mmoles/day). However, feeding was not as severely depressed. It is not known if this effect is due to changes of frequency and amplitude of reticular contractions caused by Carbocaine, since these parameters were not determined in the present experiment.

In the aforementioned experiments we have tried to characterize the feed intake response in goats and sheep to injections of volatile fatty acids and local anesthetics and functionally to locate receptor sites. Once these receptors are demonstrated beyond any doubt, it will be easier to correlate their importance with that of the volatile fatty acids produced in the rumen which are an important energy source for ruminants.

In summary, injection of acetate combined with a local anesthetic (Carbocaine) did not decrease feeding of goats or sheep during treatment days when compared to the response to acetate injected alone. When a local anesthetic (Xylocaaine) is injected near a ruminal nerve and acetate is simultaneously injected into the rumen, feeding tends to be decreased less than when acetate is injected alone. When acetate and Carbocaine are injected together into rumen pouches of goats or sheep during scheduled feedings, feed intakes are greater than when acetate is injected alone. This indicates that a neural signal instead of a humoral factor is responsible for a decrease in feeding when acetate concentration is increased in the rumen. The receptors acting in the depression of feed intake caused by propionate appear to be different by being in the ruminal veins in addition to being in the ruminal wall (3, 4). Local anesthetics injected intraruminally with propionate did not eliminate but tended to cause an even more severe depression of feed intake.

Acknowledgments

The authors thank Miss Carol McLaughlin and Mr. Othneal Clark for the technical assistance in this experiment.

This work was supported by grants-in-aid from the National Science Foundation (GB-15812) and by the Fund for Research and Teaching, Department of Nutrition, Harvard University, School of Public Health.
References