Roles of eating, rumination, and arterial pressure in determination of the circadian rhythm of renal blood flow in sheep

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ABSTRACT: To assess the roles of feeding behavior (eating and rumination) and systemic arterial pressure (SAP) on determination of the circadian rhythm of renal blood flow (RBF), 20 sheep fitted with ultrasonic flow-metering probes around both renal arteries and a submandibular balloon to monitor jaw movements (6 of them with a telemetry measurement system into the carotid artery for SAP recording), were successively assigned to 6 feeding patterns: once daily in the morning (0900 to 1100 h), afternoon (1700 to 1900 h), or evening (1900 to 2100 h); twice daily at 0900 to 1100 h and 1700 to 1900 h; ad libitum (food renewed each 2 h); and fasting (40 h). All protocols were carried out in autumn-winter, and the fasting pattern was repeated in spring-summer to evaluate the effect of the daylight length on RBF. In the once-daily feeding patterns, a rapid increase in RBF ($P < 0.05$ vs. 1-h prefeeding mean values) subsequent to the onset of meals was observed, followed by a progressive increase ($P < 0.05$), reaching a maximum 4 to 6 h after the beginning of eating, and a subsequent gradual decline until the next meal [differences vs. prefeeding values were no longer significant after 11 h (morning pattern), 13 h (afternoon pattern), and 15 h (evening pattern) from the beginning of eating]. In the twice-daily feeding pattern, each meal was also followed by an increase in RBF ($P < 0.05$ vs. prefeeding values), reaching a maximum 3 to 5 h after the onset of meals, and a posterior decline [differences vs. prefeeding values were no longer significant after 8 h (morning meal) and 5 h (afternoon meal) from the beginning of eating]. In the ad libitum feeding, no apparent rhythm in RBF was found. During fasting, a progressive reduction of RBF was observed from 2 h after the beginning of fasting ($P < 0.05$ vs. the mean value of the first fasting hour), with a slight rebound ($P < 0.05$) lasting several hours from approximately 0700 h in autumn-winter and approximately 0500 h in spring-summer. No change in the RBF profile was observed in association with rumination. Except during meals, no correlation was found between RBF and SAP. A detailed description of RBF and SAP recordings is presented. In conclusion, results showed a circadian rhythm of RBF determined by eating behavior, but not by rumination, that was independent of blood pressure and that seemed superimposed on a primary lighting-cycle-dependent RBF rhythm.

Key words: arterial pressure, circadian rhythm, eating, renal blood flow, rumination, sheep

INTRODUCTION

Circadian rhythms in renal blood flow (RBF) have been demonstrated in many omnivores and carnivores, and in most cases, a strong association with feeding (particularly with protein-rich meals) has been observed (reviewed by Premen, 1986). Little research has been done on the determination of RBF rhythm in ruminants, in spite of their particular feeding behavior (continuous feeding, rumination), end products of digestion, and metabolic pathways. In a previous report, we observed a significant 3-h increase in RBF after the morning meal in caged sheep fed once daily (Denis et al., 2004), indicating a possible influence of feeding on RBF. To confirm the association between feeding and RBF rhythm, it is necessary to monitor the RBF during imposed variations in feeding patterns (daytime, frequency, fasting).

To consider the daily variations of RBF as a specific response to feeding, an effect of meal-related systemic

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doi:10.2527/jas.2008-1386

Published December 5, 2014
arterial pressure (SAP) increase should be eliminated. In fact, Millar-Craig et al. (1978) suggested that rhythmic variations in renal hemodynamics in humans can be partly explained by the SAP circadian pattern. However, in many cases, no significant changes in SAP were observed during the increase in RBF after a meat meal in monogastric species (reviewed by Premen, 1986). To our knowledge, studies on the relationship between SAP and RBF rhythms in adult sheep are lacking. The aim of the present work was to assess the roles of feeding behavior (eating and rumination) and SAP on determination of the circadian rhythm of RBF in caged sheep.

MATERIALS AND METHODS

The experiments were performed in accordance with international ethical guidelines, and the protocol was approved by the Ethical Committee of the National Veterinary School of Lyon, France.

Animals and Diets

Twenty adult (3- to 4-yr-old) nonpregnant, nonlactating Ile de France ewes (56 to 78 kg of BW) were housed in individual pens (1 × 1.5 m) in a temperature-controlled room (18 to 22°C) with a natural lighting cycle, provided water ad libitum, and fed alfalfa pellets and hay. Owing to the gregarious nature of sheep feeding behavior, the sheep were housed 2 or 3 to a room to promote social interaction. The animals were familiarized with this procedure for 10 d before surgery.

Surgical Preparation

Transit-time ultrasonic flow-metering probes (4 mm, R-series, Transonic Systems, Ithaca, NY) were implanted bilaterally around renal arteries to allow the continuous measurement of RBF. After a 24-h fast, the sheep were premedicated with xylazine (Rompun, Bayer, Puteaux, France, 0.2 mg/kg of BW), and general anesthesia was induced with intravenous sodium thiopentone (Nesdonal, Mérial, Lyon, France, 15 mg/kg of BW) and maintained with isoflurane (Aerrane, Baxter, Maurepas, France, 0.5 to 2%) in O₂ (6 L/min). The animals were positioned successively in left and right lateral recumbence. Each flank was shaved and prepared for aseptic surgery and, through a paravertebral laparotomy, the ipsilateral kidney was located in the retroperitoneal space. The renal artery was carefully isolated from surrounding tissues, and the flow probe was placed around it and wrapped with a silicone film. Both the probe and film were sutured to the surrounding tissues with polyamide thread after having ensured that the probe was in alignment with the artery. The silicone film avoids interference of the surrounding tissues with the ultrasonic signal and contributes to immobilizing the probe in the proper position. The probe cable was then exteriorized through the surgical wound. The sheep were given amoxicillin (Clamoxyl, Beecham, St Brieuc, France, 15 mg/kg of BW i.m.) for 5 d and ketoprofen (Ketofen, Mérial, Lyon, France, 3 mg/kg of BW i.m.) for 3 d. No postsurgical disturbances were observed. The experimental protocol began at least 8 d after surgery to permit full fibrous encapsulation of the probes, as required for optimal ultrasonic signals.

In 6 of the sheep, a second procedure with the same anesthetic protocol was carried out 1 wk after the preceding surgery to implant a telemetry measurement system (Physiotel Transmitter, model TL11M3-D70-CCP, Data Sciences International, St Paul, MN) for continuous recording of SAP. The blood pressure transmitter was placed subcutaneously in the dorsal area of the neck and sutured to the surrounding tissues. The blood pressure sensor catheter was inserted into the carotid artery and secured with a polyamide thread.

At the end of the experiments, the implanted probes were validated for zero blood flow and calibrated in situ to verify the identity between the real (pumped) and the measured flows, according to the method of D’Almeida et al. (1995) and as described in our previous work (Denis et al., 2003). At the end of the procedure, animals were killed by intravenous administration of a mixture of embutramide (1 g), mebezonium (0.25 g), and tetracaine (0.025 g; T61, Intervet S.A., Beaucouze, France). If necessary, measured flows were corrected for the slight difference between the true and the read flow rate during calibration.

Recordings

Flow probes were connected to a dual-channel flowmeter (model T208, Transonic Systems), which was connected to a hardware and software system, for the acquisition and processing of biological signals (Acqknowledge III for MP150WSW, Biopac Systems Inc., Santa Barbara, CA). Mean values of RBF (right and left flows) were calculated every 10 min by the processing system. To monitor feeding behavior (eating or rumination), jaw movements were recorded with a submandibular balloon filled with polyethylene foam and connected to the processing system through a pressure transducer (model PM5, Statham Laboratories Inc., Hato Rey, Puerto Rico). For the continuous measurement of arterial blood pressure, the transmitter sent the information to a receiver (type RMC-1, Data Sciences International) connected to an interface (calibrated pressure analog adapter, type R11CPA, Data Sciences International), which transferred digital data to the Acqknowledge system; SAP was calibrated with an ambient-pressure monitor (C11PR, Data Sciences International). The processing system allowed the mean value of SAP to be calculated every 10 min and the heart rate to be calculated from SAP or RBF recordings. Renal vascular resistance (RVR) was calculated as SAP/RBF.
Feeding Patterns

Sheep were randomly and successively assigned to 6 different feeding patterns: once daily in the morning (0900 to 1100 h), afternoon (1700 to 1900 h), or evening (1900 to 2100 h); twice daily at 0900 to 1100 h and 1700 to 1900 h; ad libitum (food renewed each 2 h); and fasting (40 h). After 3 d of adaptation, each pattern was carried out on 6 to 8 sheep for at least 5 consecutive days (except fasting), simultaneously in 2 or 3 sheep in the same room. During eating time, food was available for ad libitum ingestion, and food was added as needed to ensure unrestrained voluntary ingestion. Except for the ad libitum pattern, at the end of the eating time, food was always removed. The amount ingested was monitored (by weighing the food) and data from the days of reduced ingestion (less than 60% of the current food consumption) were not considered. In the dark hours, food handling was done with minimal artificial lighting to avoid altering the natural lighting cycle. In the days preceding the fasting pattern, sheep were fed in the evening and the fasting period was considered as beginning at 0800 h. All protocols were carried out in the autumn-winter period and the fasting pattern was repeated in the spring-summer period to evaluate the effect of daylight length on RBF values.

Statistical Analyses

Data were collected with Microsoft Excel (Microsoft Excel 2000, Microsoft Corporation, Redmond, WA) software. Unless otherwise indicated, RBF and SAP values are expressed as means ± SD over 10-min periods. To compare data for RBF, SAP, and heart rate before, during, and after meals and rumination periods, an intragroup ANOVA for repeated measures was used, except when there was inequality of variance (P < 0.05; StatView, SAS Inst. Inc., Cary, NC). The a posteriori significance of intragroup contrast differences was analyzed by using Student’s paired t-test. Student’s t-test was used to compare fasting RBF data between the autumn-winter and spring-summer periods. When there was inequality of variance (P < 0.01), Friedman and Wilcoxon post hoc tests for intragroup comparisons were used. The significance threshold was set at α = 0.05, with a power of analysis of 80% for bilateral comparisons. A value of P < 0.10 was considered as a trend. Incomplete data or values apart from the 95% confidence interval were excluded from the statistical analysis.

RESULTS

Calibration of flow-metering probes showed good accuracy in the range of recorded blood flow rates. The linear regression between probe-measured and calibration pump-infused flow rates was not different from the line of identity (P < 0.05, data not shown). In most sheep, the RBF was different between kidneys, as expected from differences in size. In the results below, RBF is always the sum of left and right flows. At post-mortem examination, no macroscopic evidence of wall damage or lumen narrowing was found in any renal artery.

Different circadian rhythms of RBF were associated with each feeding pattern (Figure 1A to 1E). In the morning feeding pattern, a rapid increase in RBF subsequent to the onset of the meal was followed by a progressive increase, reaching a maximum 4 to 6 h after the beginning of eating, and a subsequent gradual decline for the next 14 to 16 h. The difference from the 1-h prefeeding mean value was significant (P < 0.05) between 0900 and 2000 h. The increase in RBF reached a maximum (24.8% of the prefeeding value) between 1220 and 1630 h. In the afternoon and evening feeding patterns, this circadian rhythm was shifted, having similar relationships with the feeding time [18.4 and 13.0% maximum increase (P < 0.05), respectively, 4 to 6 h after the beginning of eating]. In the twice-daily feeding pattern, each meal was also followed by an increase [maximum increase of 10.7% (morning) and 5.1% (afternoon; P < 0.05 vs. prefeeding values), 3 to 5 h after the beginning of eating] and a posterior decline in RBF [differences vs. prefeeding values were no longer significant after 8 h (morning meal) and 5 h (afternoon meal) from the beginning of eating]. In the ad libitum feeding pattern, no apparent rhythm in RBF was observed; values during the diurnal period (between 0700 and 1900 h) averaged 695 ± 10 mL/min, and values during the dark period (between 1900 and 0700 h) averaged 680 ± 11 mL/min. Finally, in the fasting pattern (Figure 2), a progressive reduction in RBF was observed during food deprivation, beginning 2 h after the onset of fasting (P < 0.05 vs. the mean value of the first fasting hour), with a slight regain (P < 0.05) lasting several hours from approximately 0700 h in the autumn-winter period and approximately 0500 h in the spring-summer period. After a 40-h fast, the RBF was reduced (P < 0.05) by 36.3% in autumn-winter and by 29.2% in spring-summer. The graphs presented in Figure 1A to 1E correspond to short lighting cycles (autumn-winter period).

No change in the RBF profile was observed in association with rumination periods, regardless of how long they were, the time they occurred, or the feeding pattern. Most of these periods (mainly the longest ones) appeared in the evening and during the night, concurrent with the declining phase of RBF and without disturbing its course. In a more detailed approach (Table 1), no changes were found in RBF values during medium and long rumination periods, related to pre- or postrumination values. On the contrary, during eating, a significant increase in RBF was observed, related to prefeeding values (P < 0.01 for morning and evening, and P < 0.05 for afternoon feeding patterns). This increase began abruptly: an increase in RBF was found during the first 5 min of chewing compared with the previous 5 min (600 ± 129 vs. 579 ± 130 mL/min re-
spectively, \( P < 0.01 \); means for the morning, afternoon, and evening feeding patterns).

A meal-related change in the daily profile of SAP was not found except during eating time, as illustrated in Figure 3 for the evening feeding pattern. No variation in SAP (related to prefeeding values) was observed during the significant increase in RBF subsequent to the end of a meal. Considering the successive 10-min mean values of each variable along the day, a linear regression analysis (Figure 4) showed no correlation between RBF and SAP (for each sheep individually, the \( r \) coefficient ranged from \(-0.07\) to \(0.26\), \( P > 0.10\)). During meals, the significant and rapid increase (first 5 min) in SAP lasted for the entire eating time, associated with similar increases in heart rate and RBF (Figure 3 and Table 2). Except during the first 5 min of the meal, no change in RVR was found during the first hour of feeding compared with prefeeding values (Table 2). No changes in SAP or heart rate were observed during rumination.

A detailed illustration of RBF, SAP, and rumination recordings is presented in Figure 5. As expected, kidney perfusion from renal arteries was achieved by regular 2-wave flow pulses, with the main wave, associated with the SAP systolic pulse, preceding the smaller one, which appeared during the diastolic phase of the SAP. The extreme values of RBF pulses oscillated from \(+55.5 \pm 10.1\) to \(-25.9 \pm 4.0\)% of the mean value. During rumination, before each chewing period (concurrent with the regurgitation of the ruminal bolus), a rapid and transient decline in RBF was observed (42.5 \pm 11.0\% less than the previous 1-min mean minimal flow, \( P < 0.01\)). This event was always present in all sheep and on both renal arteries, occurring 1.6 \pm 0.4 s before the beginning of the first chewing movement (1,000 measurements, 50 \times 20 sheep). It was not found at any other moment of the day. The synchrony of this event with a similar decline in SAP (13.9 \pm 3.3\% less than the previous 1-min mean diastolic pressure, \( P < 0.01\)) was evident in sheep provided with telemetry transmitters.

**DISCUSSION**

Transit-time flow probes are widely used for the measurement of regional blood flow in domestic animals. In adult sheep, the system was successfully used previously for chronic RBF measurements over many months (Bednarik and May, 1995). The technique provided reliable and accurate data, allowing a long-lasting, continuous, and bilateral recording of blood flow in renal arteries without stressing the animal. Moreover, this technique enabled the recording of transient and rapid flow variations, which are impossible to detect by classical clearance methods.

The results showed a RBF circadian rhythm determined by the feeding pattern: a long-lasting increase after feeding, regardless of the time of the meals. The stimulatory effect of the meal on renal perfusion was also concluded from the decreasing RBF values during fasting, and from the high constant RBF values during the more continuous diurnal feeding in the ad libitum pattern. The profile of RBF changes after the morning meal was similar to that described in a previous work (Denis et al., 2004). A similar circadian rhythm, dependent on feeding time, was reported in another herbivore species, the rabbit, by using chronic implants of ultrasonic flow probes around the renal arteries (Barrett et al., 2001). These authors observed an increase in RBF beginning with the onset of feeding (0900 to 1100 h), reaching maximum values at approximately midday, and progressively decreasing over the next 12 h. When the feeding time was shifted to 1500 h, the RBF profile

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**Figure 1.** Change in renal blood flow (RBF, mL/min, both kidneys) over 24 h in 5 feeding patterns: once daily (A, morning; B, afternoon; C, evening), twice daily (D), and ad libitum (E). Horizontal bars = feeding time. \(* P < 0.05\) compared with the 1-h prefeeding mean value. Values = means over 10 min, \(n = 6\) to 8 sheep (\(\times 5\) d each) for each pattern.
also shifted. The existence of a circadian RBF rhythm independent of feeding behavior is consistent with the report of Braaksma et al. (2000) in fetal sheep, in which rhythmic variations of fetal renal perfusion during a 24-h period were not attributable to the ad libitum feeding protocol of the mother. Those authors recognized that the origin of the fetal RBF rhythm was not known. Nevertheless, the absence of an association between RBF and feeding during fetal life does not mean that this association could not be present in the adult; currently, biorhythms that develop, mature, or adjust after birth are known to exist and have been attributed to the intervention of external triggers (reviewed by Anders, 1982).

No effect of rumination on RBF was observed. Because rumination is considered a “second meal,” inducing a regain in digestive and absorptive processes without new ingested food, it is not clear why it did not influence RBF. Even during rumination, RBF values were not different from pre- or postrumination values. In contrast, ingestion was concurrent with a rapid and sustained increase in RBF. Thus, what is the role of chewing, if any, in the genesis of the RBF increase during meals? Because rumination did not elicit such a response, it may be that the increase in RBF during meals is SAP dependent, because SAP and heart rate were also increased from the beginning of the meal, but did not change during rumination. Moreover, the unchanged RVR during the first hour of meals indicates that RBF passively followed the increase in SAP (the initial 5-min elevation in RVR could be attributed to involvement of the sympathetic autonomous system, as suggested by the increased heart rate). Nevertheless, the participation of meal chewing in the origin of an oral reflex that increased RBF cannot be excluded: it is well known that the acceleration of the forestomach motility elicited by ingestion (oral reflex of the reticulum; Borgatti and Matscher, 1958) is not present during rumination.

Our results clearly indicated that the postprandial increase in RBF was independent of the SAP. In addition, Braaksma et al. (2000) showed, in fetal sheep, 24-h sustained increase in RBF. Thus, what is the role of chewing, if any, in the genesis of the RBF increase during meals? Because rumination did not elicit such a response, it may be that the increase in RBF during meals is SAP dependent, because SAP and heart rate were also increased from the beginning of the meal, but did not change during rumination. Moreover, the unchanged RVR during the first hour of meals indicates that RBF passively followed the increase in SAP (the initial 5-min elevation in RVR could be attributed to involvement of the sympathetic autonomous system, as suggested by the increased heart rate). Nevertheless, the participation of meal chewing in the origin of an oral reflex that increased RBF cannot be excluded: it is well known that the acceleration of the forestomach motility elicited by ingestion (oral reflex of the reticulum; Borgatti and Matscher, 1958) is not present during rumination.

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**Figure 2.** Change in renal blood flow (RBF, mL/min, both kidneys) during a 40-h fast. (●) Autumn-winter period; (○) spring-summer period. *P < 0.05 compared with the mean value of the first hour (0800 to 0900 h) of fasting. a = P < 0.05 between periods. Values = means over 10 min (n = 6 sheep for each period).

**Table 1.** Mean values of renal blood flow (expressed as a percentage of the 30-min prerumination or prefeeding mean values) during the rumination periods, 1 the 30 min postrumination, 2 the first hour of feeding, and the 30 min postfeeding for the different feeding patterns

<table>
<thead>
<tr>
<th>Feeding pattern</th>
<th>Ruminatin</th>
<th>Postrumination</th>
<th>Feeding</th>
<th>Postfeeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0900 to 1100 h</td>
<td>99.4 ± 1.7</td>
<td>99.9 ± 2.8</td>
<td>109.3 ± 4.9**</td>
<td>112.5 ± 9.5**</td>
</tr>
<tr>
<td>1700 to 1900 h</td>
<td>99.9 ± 1.7</td>
<td>99.4 ± 0.8</td>
<td>107.8 ± 3.9*</td>
<td>104.6 ± 3.6*</td>
</tr>
<tr>
<td>1900 to 2100 h</td>
<td>101.3 ± 1.5</td>
<td>99.9 ± 2.1</td>
<td>107.0 ± 2.1**</td>
<td>100.4 ± 7.6</td>
</tr>
<tr>
<td>Ad libitum</td>
<td>98.9 ± 2.4</td>
<td>98.1 ± 4.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fasting</td>
<td>101.0 ± 2.4</td>
<td>99.0 ± 0.8</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1Mean value of 3 periods (40 to 90 min each) by sheep.
2Following the rumination periods considered.
*P < 0.05 and **P < 0.01 vs. prerumination or prefeeding values, n = 8 sheep (× 2 d each) for each pattern.
rhythmic variations in RBF that were independent of the rhythm of the mean arterial pressure. Gehrig et al. (1986) reported in rats that the RBF increase induced by feeding was independent of the mean arterial pressure. In humans, Voogel et al. (2001) reported that the circadian variation of the effective renal plasma flow or glomerular filtration rate (as measured by clearance methods) did not result from changes in blood pressure or cardiac output. In the work of Barrett et al. (2001) in rabbits, the circadian RBF profile seemed blood pressure dependent, because both variables increased at the same time and had the same profile. Nevertheless, a simultaneous decrease in renal resistance was observed by these authors, in spite of an increased activity of the renal sympathetic nerves, indicating that the mechanism responsible for the increase in RBF may be independent of blood pressure.

For a better characterization of the role of meals in the genesis of the RBF rhythm, the eventual participation of the circadian lighting cycle in the extent of the renal perfusion should not be neglected. Under our experimental conditions, the way to evaluate this influence was to impede eating and thereby suppress the effect of feeding on RBF. Thus, the fasting pattern was repeated during the long lighting cycles (spring-summer) to compare RBF changes with the short lighting cycles (autumn-winter). The partial regain in RBF after approximately 20 h or more of fasting may be attributable to the influence of the lighting cycle, given that its onset was coincident, in both protocols, with the breaking of dawn. In spring-summer, the dawn is advanced, and in this period, the regain in RBF appeared to shift toward earlier hours than in the autumn-winter protocols. Likewise, the shorter lighting period could be responsible for the greater percentage of RBF reduction at the end of fasting found in the autumn-winter protocols. Some facts observed in the other feeding patterns also may indicate the influence of the lighting cycle on RBF. The increase in RBF values between 0800 and 1000 h in the afternoon and evening feeding patterns and its significant diminution between 0400 and 0700 h in the morning feeding pattern seemed to be changes that were independent of the feeding behavior. In addition, the percentage of postprandial maximum RBF increase diminished as the meals were shifted from the morning, with ample natural light, to the afternoon and evening hours, with very poor natural lighting, as protocols were performed in the autumn-winter period.

**Table 2.** Comparison of the mean values of renal blood flow (RBF), systemic arterial pressure (SAP), renal vascular resistance (RVR), and heart rate (HR) during the hour preceding and the hour following the beginning of feeding, and during the 5-min prefeeding and the first 5 min of feeding in the evening feeding pattern

<table>
<thead>
<tr>
<th>Period</th>
<th>RBF, mL/min</th>
<th>SAP, mmHg</th>
<th>RVR</th>
<th>HR, bpm&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-h prefeeding</td>
<td>584 ± 51</td>
<td>82 ± 11</td>
<td>0.141 ± 0.087</td>
<td>70 ± 19</td>
</tr>
<tr>
<td>First hour of feeding</td>
<td>619 ± 66**</td>
<td>90 ± 13*</td>
<td>0.145 ± 0.094</td>
<td>96 ± 14**</td>
</tr>
<tr>
<td>5-min prefeeding</td>
<td>592 ± 75</td>
<td>83 ± 10</td>
<td>0.142 ± 0.083</td>
<td>70 ± 18</td>
</tr>
<tr>
<td>First 5 min of feeding</td>
<td>610 ± 55*</td>
<td>97 ± 15**</td>
<td>0.162 ± 0.144*</td>
<td>95 ± 22**</td>
</tr>
</tbody>
</table>

<sup>1</sup>Beats per minute.

*P < 0.05 and **P < 0.01, feeding vs. prefeeding, n = 6 sheep (× 2 d each).

**Figure 3.** Change in renal blood flow (RBF, mL/min, both kidneys) and systemic arterial pressure (SAP, mmHg) over 24 h in the evening feeding pattern. Horizontal bar = feeding time. *P < 0.05 compared with 1-h prefeeding mean values. Values = means over 10 min (n = 6 sheep).
In the twice-daily feeding pattern, these percentages were less than the corresponding ones in the once-daily feeding, probably because of more frequent meals. Therefore, the meal-dependent RBF circadian rhythm seemed to be superimposed on basal RBF levels determined by the lighting cycle. The RBF could also be affected by sheep posture (lying or standing) or attitude (somnolence or awakening), but these behaviors are influenced to a large extent by the lighting cycle itself. More specific protocols should be carried out to study these possibilities.

Our phasic RBF signals were similar to those recorded by Bednarik and May (1995) from the left renal arteries of sheep, using the same transit-time flow probes. These authors reported flow values reasonably similar to ours. The 2-wave flow pulses are determined by the arterial pressure gradient (main wave) and the reflected pressure waves at the aortic and renal artery branching point, which increase the renal perfusion during the diastolic decline of the SAP (small wave; reviewed by Nichols and O'Rourke, 1998). The declines in SAP and RBF before rumination were synchronous, despite the important distance between the 2 recording points (carotid and renal arteries). There was no evidence in the SAP tracing to indicate a cardiac cause (i.e., heart rate and stroke volume were unchanged, and there was no lengthening of the diastolic phase leading to a punctual decline in SAP) or a change in the peripheral vascular resistance (only one pulse was affected each time). More likely, both declines were caused by the short, sharp, negative intrathoracic pressure produced by the inspiration effort against a closed glottis, which initiates regurgitation (aspiration of the rumen content into the thoracic esophagus). This negative pressure may reduce the transmural tension of the intrathoracic aorta, producing an acute decompression of the vessel and a short decline in carotid artery pressure and RBF. In fact, the more abrupt descending pressure slope leading to the acute decline should be expected if the aortic impedance is suddenly reduced. The intrathoracic negative pressure occurs regularly 1 to 2 s before the first chewing movement (Stevens and Sellers, 1960), coincident with the decreases in SAP and RBF. The regurgitation antiperistaltic wave could also affect the aortic impedance, owing to the contact between the aorta and the esophagus in the sheep thorax (May, 1964), but this cause is unlikely because the decreases were not observed during the deglutition peristalsis.

In conclusion, these results showed a circadian rhythm of RBF determined by eating behavior, but not by rumination, that was independent of blood pressure and that seemed superimposed on a primary lighting cycle.
dependent RBF rhythm. It should be kept in mind that our experimental feeding patterns are not the natural ones for ruminant species. Thus, the observed feeding-dependent RBF circadian rhythm would be expected to be less obvious in grazing conditions (pasture-based extensive breeding systems), such as in our ad libitum pattern, because ruminants typically graze continuously. Possible links between meals and the postprandial increase in RBF (e.g., digestion end products absorbed, hormonal background, vasoactive agents) need to be further investigated in ruminants.

LITERATURE CITED


