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The Histamine H_1 Receptor Is Not Involved in Local Control of Mammary Blood Flow in Dairy Cows

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ABSTRACT

Low concentrations of the essential amino acid histidine in circulation have been shown to increase mammary blood flow and it has been suggested that this effect is mediated by histamine. The hypotheses tested in this experiment were that interstitial histamine concentrations in the mammary gland are related to arterial His concentrations and that mammary blood flow is reduced by extracellular histamine via H_1 receptors. The hypotheses were tested by infusing saline or chlorpheniramine, a blocker of the H_1 histamine receptor, into the arterial supply of the mammary glands of lactating cows infused with 44 g/h of amino acid mixtures with or without His for 10 h. Infusates were administered in a 2×2 factorial arrangement within a 4×4 Latin square to 4 multiparous Holstein cows in mid lactation. Exclusion of His from the infusate decreased protein content in milk from the infused udder half from 3.98 to 3.77%, and increased arterial α-amino-nitrogen concentration from 3.2 to 3.4 m*M*. Neither the decreased arterial His concentration nor the H_1 blocker affected plasma flow to the infused udder half. We conclude that histamine is not involved in the regulation of mammary blood flow. The H_1 blocker decreased milk production in the infused udder half from 4.6 to 3.5 kg without affecting protein, fat, and lactose percentages, suggesting an inhibition of milk ejection. Cows on chlorpheniramine ate less feed during the infusion than saline-infused cows, which resulted in lower arterial concentrations and mammary uptakes of acetate. The efficiency of plasma triacylglycerol uptake across the mammary glands was decreased by chlorpheniramine but net uptake of long-chain fatty acids was not affected. The mechanism by which an amino acid deficiency influences mammary blood flow does not involve histamine signaling through the H_1 receptor and remains unidentified.

Key words: histidine, histamine, mammary plasma flow, dairy cow

INTRODUCTION

To sustain milk synthesis the mammary gland depends on an adequate vascular supply of nutrients, where mammary blood flow is thought to play an important role (Davis and Collier, 1985). In general, mammary blood flow correlates closely with milk production in ruminants (Linzell, 1974; Nielsen et al., 1990), which indicates that mammary blood flow, like flow to other tissues, is related to the metabolic activity of the mammary gland (Prosser et al., 1996). By extension, if circulating concentrations of milk precursors affect metabolic activity of the mammary gland, then they should also affect its rate of blood flow. When circulating concentrations of glucose were increased from 3.6 to 6.4 m*M* by close arterial infusion for 10 h, mammary plasma flow decreased by 16% (Cant et al., 2002). Similarly, when circulating concentrations of the essential amino acid His were reduced from 73 to 8 μ*M* by abomasal infusion of a His-lacking amino acid mix for 6 d, mammary plasma flow increased by 33% (Bequette et al., 2000).

Blood flow rate depends on vascular resistance to the arteriovenous pressure gradient. Locally produced compounds such as adenosine, CO_2, prostaglandins, and NO have been shown to relax the resistance to blood flow in the mammary glands (Linzell, 1974; Nielsen et al., 1995; Lacasse et al., 1996). It has been proposed that the nutrient effect on mammary blood flow is related to the interstitial release of such vasoactive agents as a consequence of over- or underproduction of ATP relative to its utilization (Cant and McBride, 1995; Cant et al., 2003). According to this ATP balance hypothesis, Cant et al. (2003) suggested that decreased plasma concentrations of the energy metabolites acetate and β-hydroxybutyrate, characteristic of an amino acid defi-

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ciency in lactating cows (Weekes et al., 2006), could account for the observed hyperemia in His-deficient goats (Bequette et al., 2000). However, energy metabolites and mammary blood flow have not been measured simultaneously in the same His-deficient animals, so the explanation remains tenuous at best.

An alternative explanation of the low His-induced hyperemia is due to local production of histamine, as histamine has been shown to have vasoactive effects in several tissues (Buckley et al., 1988; Parsons et al., 1992). Histamine can be both vasoconstrictive and vasodilatory. Vasodilation appears to be due to release of relaxation factors from the vascular endothelium upon activation of either H_1 or H_2 receptor (Jin et al., 2006; Stähli et al., 2006). Vasoconstriction, on the other hand, is mediated in the absence of endothelium by H_1 receptors (Satoh and Inui, 1984; Jin et al., 2006; Stähli et al., 2006). At low concentrations, histamine dilated mammary arteries from humans in vitro, but constricted at greater concentrations (Stähli et al., 2006). At all concentrations, histamine exerted contractile effects on mammary arteries from nonlactating goats (Jakobsen et al., 1994). The enzyme for synthesis of histamine from His, histamine decarboxylase, has been detected in mast cells and epithelial cells of the bovine and murine mammary gland (Maslinski et al., 1993). If histamine release into the mammary interstitium is directly related to arterial His concentration and histamine is constrictive to mammary blood flow, then a temporary His deficiency would be expected to increase mammary blood flow.

The hypotheses tested in this experiment were that interstitial histamine is related to arterial His concentration and that mammary blood flow is reduced by extracellular histamine via H_1 receptors. The hypotheses were tested by infusing chlorpheniramine (**CPA**), a blocker of the H_1 histamine receptor, into the arterial supply of the mammary glands of lactating cows given amino acid mixtures with or without His for 10 h. If His is constrictive to mammary blood flow via H_1-receptors, then CPA should increase flow.

MATERIALS AND METHODS

All animal procedures and holding facilities were approved by the Animal Care Committee at the University of Guelph. Four second-lactation Holstein cows at 223 ± 20 DIM and averaging 606 ± 11 kg of BW and 30 ± 1 kg/d milk were assigned to the present experiment. The cows were fitted with polyurethane catheters in both subcutaneous mammary abdominal veins and with polyethylene catheters in both external iliac arteries via the saphenous arteries. The external iliac position was confirmed by injection of Evans blue into the arterial catheter (Cant et al., 2001).

Table 1. Chemical composition of feedstuffs

Item	Concentrate	Hay	TMR
Composition of TMR, % of as fed	44	56	
DM, % of as fed	87.2	90.0	88.8
CP, % of DM	11.2	16.9	14.4
Buffer solubility, % of CP	24.8	47.6	40.0
NDF insolubility, % of CP	5.8	16.4	12.9
ADF insolubility, % of CP	1.8	8.5	6.3
NDF, % of DM	10.4	53.3	34.8
ADF, % of DM	3.3	43.1	25.9
Cellulose, % of DM	3.3	36.1	21.9
Lignin, % of DM	0.0	7.0	4.0
Fat, % of DM	8.1	0.7	3.9
Ash, % of DM	4.4	8.3	6.6
Ca, % of DM	0.56	0.80	0.71
P, % of DM	0.47	0.30	0.38
Mg, % of DM	0.17	0.20	0.19
Calculated NE_L, Mcal/kg of DM[1]	2.06	1.20	1.57

[1]Calculated according to NRC (2001).

Cows were housed in tie stalls bedded with wood shavings. They were milked and fed a TMR low in protein at 0800 and 1800 h daily. The ration was composed of 44% concentrate (85% corn, 5% soybean meal, 5% tallow, and 5% mineral mix on a DM basis) and 56% chopped, mixed hay (Table 1). Concentrate and hay were sampled at each feeding and composited for analysis at a commercial laboratory. Before every feeding, orts were removed and weights recorded. Milking was performed with a modified bucket milker to collect milk from each udder half separately.

The experiment was conducted in 2 blocks, with 2 cows in each block, because the facilities only allowed for sampling from 2 cows at a time. Each cow was randomly assigned to 4 treatments on 4 consecutive days in a balanced 4 × 4 Latin square design. The 4 treatments were a 2 × 2 factorial arrangement of arterial infusions of complete and His-lacking AA mixtures with or without CPA. Amino acids were purchased from US-Biochemical (Cleveland, OH), *para*-aminohippuric acid (**PAH**) was purchased from ICN Biochemicals (Aurora, OH), and CPA from Sigma Chemical Co. (Oakville, Ontario, Canada). Solutions of AA (147 g/L) and CPA (3 g/L saline) were prepared the day before infusion. All solutions were adjusted to pH 7.4 and filtered for sterility through a 0.22-μm cellulose acetate filter into autoclaved polypropylene bottles. Amino acid solutions were infused for approximately 10 h between morning and evening milking through sterile Tygon surgical tubing into one iliac artery at 5.0 ± 0.2 mL/min. Every 30 min, either saline or the CPA solution was infused at 5.0 ± 0.2 mL/min for 5 min into the same artery as the AA. Infusion rates were measured by recording weights of infusate bottles several times during the day. The AA

infusion rate amounted to 44 g/h, and the infusion of CPA amounted to 150 mg/h.

Iliac plasma flow rate was estimated either from the arteriovenous concentration difference (**AVD**) of PAH included in the AA infusates at 16.6 g/L (Cant et al., 2002), or from the exponential decline of venous PAH concentration following a rapid injection of approximately 100 mg of PAH into the external iliac artery according to Qiao et al. (2005). In either case, at 8, 9, and 10 h into the infusion, blood samples were withdrawn with a syringe pump from the external iliac artery contralateral to infusion and the ipsilateral venous catheter simultaneously over a period of 10 min. We used second-lactation cows to avoid the reversal of blood flow in the pudendal veins that contaminates the mammary arteriovenous difference in cows of third lactation or higher (Thivierge et al., 2000). Venous anastomoses between right and left udder halves could also contaminate the unilateral arteriovenous difference but we have observed negligible crossover previously (Cant et al., 2001), and Metcalf et al. (1992) reported little or no crossover in cows when standing. Samples were collected from standing cows into EDTA Vacutainers (Becton Dickinson, Franklin Lakes, NJ) on ice. Plasma was separated by centrifugation for 20 min at 1,500 $\times g$ and 4°C, transferred to polypropylene tubes, and stored at –20°C until analyzed. Milk samples were collected at the evening milking from each udder half and stored at 5°C for no more than 2 d before analysis.

Concentrations of PAH in plasma and infusates were measured as described previously (Cant et al., 2001). Arterial and venous samples from each cow were pooled daily and analyzed for glucose (Sigma kit no. 510-A; Raabo and Terkildsen, 1960), triacylglycerol (**TAG**; Sigma kit no. 336; McGowan et al., 1983), acetate (Boehringer Mannheim kit; R-Biopharm GmbH, Darmstadt, Germany; Bergmeyer and Moellering, 1983), BHBA (Cant et al., 1993), NEFA (NEFA C kit; Wako Chemicals GmbH, Neuss, Germany; Johnson and Peters, 1993), urea (Sigma kit no. 640-B; Chaney and Marbach, 1962), and α-amino N (**AAN**; Evans et al., 1993). Long-chain fatty acid concentrations were calculated as 3 × TAG (μM) + NEFA (μM). Protein, fat, and lactose contents of milk samples were measured by infrared spectroscopy (AOAC, 1996).

Iliac plasma flow (L/h) was calculated as infusion rate of PAH (mg/h)/AVD of PAH (mg/L), or as dose of PAH (mg)/area under venous PAH curve (mg·h/L). Local mammary arterial concentrations of AAN were calculated as systemic arterial concentration + infused AAN/iliac plasma flow, and AVD of AAN was calculated as the difference between milk vein and local arterial concentrations. Mammary extraction percentages of blood metabolites were calculated as the ratio of AVD to arterial concentration. A change in extraction can arise from a change in the rate constant for net uptake from the capillary, k, or the volume of perfused capillaries, $Vol_{cap}{\cdot}N_{cap}$, due to local vasodilatory mechanisms. Thus, a $k{\cdot}Vol_{cap}{\cdot}N_{cap}$ product was calculated as –ln(1 – extraction) × iliac plasma flow (Cant and McBride, 1995).

Variance in observations Y_{ijklm} was analyzed by the GLM procedure of SAS version 9 (SAS Institute Inc., Cary, NC) according to

$$Y_{ijklm} = \mu + block_i + cow(block)_{j(i)} + per_k + his_l + cpa_m + his \times cpa_{lm} + \varepsilon_{ijklm}$$

where μ = overall mean, $block_i$ = ith effect of block (i = 1 or 2), $cow(block)_{j(i)}$ = jth effect of cow within block (j = 1 or 2), per_k = kth effect of period (k = 1 to 4), his_l = lth effect of infused His level (l = 1 or 2), cpa_m = mth effect of the histamine H_1 receptor inhibitor (m = 1 or 2), his × CPA_{lm} = effect of interaction between the 2 main effects, and ε_{ijklm} = random variation, assumed to be $N(0, \sigma^2)$. Because of missing observations of metabolite concentrations on d 1 for one of the cows, results were expressed as least squares means. Statistical significance was declared at $P < 0.05$ and trends were declared at $0.05 < P \leq 0.15$. To test for treatment carryover effects from one day to the next in the Latin square design, the previous day's treatment was included as a covariate in the ANOVA model. None of the previous day covariate effects were significant.

RESULTS

The profile of the AA infusate and the calculated increase in AA concentrations of the blood supplying the infused half of the mammary gland are presented in Table 2. Based on measured rates of iliac plasma flow, the infusion increased the local mammary mean arterial plasma concentrations of essential AA and nonessential AA by 446 and 670 μM, respectively.

Subtraction of His from the AA infusate had no effect on milk or component yields, fat or lactose percentages, but milk protein content tended to decrease from 3.98 to 3.79 ($P = 0.07$) on average (Table 3). The histamine H_1 receptor inhibitor CPA decreased feed intake by 20% ($P = 0.02$) during the infusion and tended to decrease milk, protein, fat, and lactose yields ($0.05 < P < 0.10$). There was no effect of CPA on the proportions of protein, fat, and lactose in milk. No interactions between His and CPA infusion were observed in the infused udder half.

Neither of the infusion treatments affected iliac plasma flow (Table 4). Systemic arterial concentrations of AAN tended to increase from an average of 3.18 to 3.42 mM ($P = 0.09$) when His was removed from the

Table 2. Amino acid composition of infusate and estimated increase in mammary plasma AA concentrations at a plasma flow rate of 322 L/h

AA	g/L	Local increase in plasma concentration, μM
Ala	4.55	53
Arg-HCl	4.84	24
Asp	10.56	82
Cys	1.32	8
Glu	28.60	202
Gly	2.64	37
His-HCl[1]	3.80	22
Ile	7.77	62
Leu	13.20	105
Lys-HCl	11.59	57
Met	3.52	25
Pro	14.52	131
Ser	8.07	80
Thr	7.04	61
Trp	2.20	11
Tyr	13.49	77
Val	8.95	79
Essential AA	63	446
Nonessential AA	84	670
HCl	1.0	
NaOH	10.0	

[1]His was not present in all infusates.

infusate, but close arterial concentrations, extraction percentages, and uptakes were not affected. Histidine subtraction tended to increase mammary long-chain fatty acid uptake from 73 to 86 mmol/h ($P = 0.15$). Infusion of CPA decreased arterial and venous concentrations of acetate by 25 ($P = 0.02$) and 35% ($P = 0.01$), respectively, and extraction increased from 76 to 80% ($P = 0.01$). There was no effect of CPA on the $k \cdot Vol_{cap} \cdot N_{cap}$ for acetate, indicating that the dynamics of acetate uptake were not affected by treatment. Net uptakes of acetate, in mmol/h, were 23% lower during CPA infusion ($P = 0.05$) because of the lower acetate concentrations in arterial plasma. The $k \cdot Vol_{cap} \cdot N_{cap}$ for TAG and long-chain fatty acids tended to be decreased by CPA ($0.05 < P < 0.10$).

DISCUSSION

Role of Histamine in Mammary Blood Flow

The hypothesis under test in this experiment consisted of 2 parts: first, that histamine release into the interstitial fluid surrounding the mammary vasculature is directly related to arterial His concentration, and second, that mammary blood flow is reduced by interstitial histamine via H_1 receptors. According to this hypothesis, the effect of blocking the H_1 receptor with CPA should be an increase in mammary blood flow. The effect of a reduced arterial His concentration should also be an increase in mammary blood flow. No effect of treatment on iliac plasma flow was observed, so the hypothesis is rejected.

A decrease in arterial His concentration when His was subtracted from the infusion of 44 g/h of AA was evidenced by the 0.22-percentage-unit decrease in milk protein content, which is similar in magnitude to the 0.16-unit decrease observed when His was subtracted from 30 g/h of the same infusate (Cant et al., 2001). No hyperemic response to the low His concentration was observed in the previous experiment (Cant et al., 2001) or in the current one. The results suggest that if histamine is indeed vasoactive in the mammary vasculature it is not released in proportion to concentration of its precursor, His.

Histamine is synthesized from His by histidine decarboxylase, and the rate of histamine synthesis is there-

Table 3. Least squares means of feed intake, milk yield, and milk contents from the infused udder half after 10 h of infusion of an AA solution with or without His, and saline or a solution of the histamine H_1 receptor inhibitor chlorpheniramine (CPA), into one external iliac artery of 4 cows

	Treatment							
	No His		His			P-value[1]		
Item	No CPA	CPA	No CPA	CPA	SEM	His	CPA	His × CPA
Feed intake, kg/d	17.7	14.1	18.2	14.5	1.1	0.71	0.02	0.94
Milk yield, g/h	545	427	539	400	57	0.78	0.06	0.86
Protein								
%	3.86	3.68	3.99	3.98	0.10	0.07	0.35	0.39
g/h	19.4	14.9	20.0	15.8	2.1	0.74	0.09	0.95
Fat								
%	4.41	4.32	4.50	4.45	0.27	0.68	0.81	0.94
g/h	22.3	18.6	23.2	17.7	2.0	1.00	0.06	0.65
Lactose								
%	4.32	4.49	4.36	4.41	0.10	0.81	0.31	0.55
g/h	23.4	19.2	23.4	17.6	2.4	0.76	0.08	0.75
Protein:fat	0.87	0.86	0.89	0.94	0.04	0.33	0.73	0.49

[1]Probability of no effect of infusion of His, CPA, or their interaction.

Table 4. Least squares means of plasma flows and balance of plasma metabolites across the infused udder half of 4 cows during the last 3 h of a 10-h infusion of an AA solution with or without His, and saline or a solution of the histamine H_1 receptor inhibitor chlorpheniramine (CPA), into one external iliac artery

	Treatment							
	No His		His			*P*-value[1]		
Item	No CPA	CPA	No CPA	CPA	SEM	His	CPA	His × CPA
Plasma flow, L/h	337	326	330	298	17	0.38	0.29	0.60
Glucose								
Artery, m*M*	3.43	2.77	3.58	3.38	0.31	0.31	0.25	0.51
Vein, m*M*	2.66	2.08	2.58	2.54	0.18	0.39	0.18	0.23
Extraction, %	21.7	25.5	28.2	25.0	3.3	0.44	0.93	0.37
$k \cdot Vol_{cap} \cdot N_{cap}$,[2] L/h	94.1	94.1	109	87.6	11.7	0.76	0.44	0.44
Uptake, mmol/h	282	234	310	255	43	0.61	0.31	0.94
Acetate								
Artery, m*M*	1.10	0.85	1.19	0.86	0.08	0.58	0.02	0.68
Vein, m*M*	0.25	0.18	0.30	0.18	0.02	0.40	0.01	0.23
Extraction, %	77.3	79.1	74.8	81.0	0.9	0.79	0.01	0.08
$k \cdot Vol_{cap} \cdot N_{cap}$, L/h	496	497	455	492	34	0.55	0.63	0.65
Uptake, mmol/h	288	229	285	210	24	0.68	0.05	0.76
BHBA								
Artery, m*M*	1.35	1.42	1.39	1.28	0.19	0.86	0.83	0.91
Vein, m*M*	0.75	0.89	0.81	0.66	0.10	0.46	0.99	0.23
Extraction, %	42.4	38.5	40.7	47.5	4.0	0.44	0.74	0.27
$k \cdot Vol_{cap} \cdot N_{cap}$, L/h	190	154	174	194	21	0.62	0.74	0.29
Uptake, mmol/h	202	176	195	197	43	0.89	0.80	0.78
Triacylglycerol								
Artery, μ*M*	140	142	137	137	13	0.81	0.94	0.96
Vein, μ*M*	66	77	69	72	7	0.92	0.44	0.64
Extraction, %	53.0	45.0	51.3	49.3	3.6	0.75	0.26	0.47
$k \cdot Vol_{cap} \cdot N_{cap}$, L/h	276	199	250	210	23	0.78	0.06	0.50
Uptake, mmol/h	26.0	21.9	22.7	19.7	3.0	0.42	0.32	0.87
NEFA								
Artery, μ*M*	135	144	117	137	18	0.57	0.50	0.80
Vein, μ*M*	94	101	93	99	11	0.95	0.60	0.96
Long-chain fatty acids								
Artery, μ*M*	554	568	529	549	35	0.58	0.66	0.94
Vein, μ*M*	292	331	301	315	25	0.90	0.37	0.67
Extraction, %	48.4	42.3	45.5	45.7	3.1	0.95	0.42	0.39
$k \cdot Vol_{cap} \cdot N_{cap}$, L/h	238	184	214	191	17	0.65	0.09	0.45
Uptake, mmol/h	92.3	79.7	76.5	69.8	7.0	0.15	0.26	0.71
α-Amino N								
Opposite artery, m*M*	3.54	3.30	3.14	3.21	0.11	0.09	0.49	0.25
Artery, m*M*	4.66	4.34	4.25	4.36	0.11	0.17	0.43	0.14
Vein, m*M*	3.87	3.87	3.54	3.66	0.22	0.30	0.83	0.81
Extraction, %	16.7	10.6	16.4	16.4	4.1	0.56	0.52	0.53
$k \cdot Vol_{cap} \cdot N_{cap}$, L/h	66.8	40.0	61.0	54.9	16.3	0.81	0.39	0.58
Uptake, mmol/h	283	167	229	204	58	0.91	0.32	0.50
Urea								
Artery, m*M*	3.52	3.59	3.12	3.45	0.40	0.56	0.67	0.77

[1]Probabilities of no effect of infusion of His, CPA, or their interaction.

[2]$k \cdot Vol_{cap} \cdot N_{cap} = -Ln\ (1 - \text{extraction}) \times$ iliac plasma flow (Cant and McBride, 1995).

fore expected to follow a dependence on substrate concentration, but histamine can be stored intracellularly before release. Most of the histamine stored in the body is in mast cells and basophils of the immune system. The majority of histamine in the mammary glands is also located in mast cells but histamine and histidine decarboxylase are also present in the mammary epithelium (Maslinski et al., 1993; Wagner et al., 2003). Upon stimulation of release, dependent on the cell type and anatomical location, histamine acts in various roles as a neurotransmitter or a signal of inflammation and other immune responses, gastric acid secretion, or smooth muscle contraction. After a week of dosing rats orally with 1 g/kg of His twice daily, histamine concentrations in regions of the brain were significantly elevated, but concentrations of the metabolite *tele*-methylhistamine, indicating histamine release, were not affected (Lozeva et al., 2003). Thus, it appears that release of histamine is a stronger controller of interstitial histamine concentration than concentration of His. Plasma histamine concentrations were also not affected by the His dosing (Lozeva et al., 2003). These observa-

tions support rejection of the hypothesis that a reduced arterial His concentration affects histamine concentration in the mammary vasculature of the lactating dairy cow.

The lack of a blood flow response to the H_1 receptor blocker CPA allows us to reject the second part of the hypothesis that histamine is vasoactive in the mammary vasculature via H_1 receptors. Histamine has been shown to exert a combination of constrictive and dilatory effects on various arteries, each of which are mediated through separate response mechanisms. There are 4 histamine receptors, designated H_1 to H_4 (Wagner et al., 2003). Antagonists of either the H_1 or H_2 receptor, but not both in the same artery, have abolished the vasodilatory effect of histamine (Jin et al., 2006; Stähli et al., 2006), which is dependent on precontraction of the artery and the presence of an intact endothelium (Jin et al., 2006; Stähli et al., 2006). The net effect of histamine on isolated arteries at greater concentrations is constrictive. The vasoconstriction occurs in the absence of endothelial cells but is abolished by analogs of the H_1 receptor (Jin et al., 2006; Stähli et al., 2006). The vasoconstriction is thus considered to be due to a direct effect of histamine on the arterial smooth muscle or vascular nerves. Internal mammary arteries of the human were found to be dilatory to histamine at low concentrations, whereas radial arteries were not (Stähli et al., 2006). Both constricted at higher histamine concentrations. The pudendal arteries of nonlactating goats only responded to histamine with vasoconstriction (Jakobsen et al., 1994). In addition, constriction of the caprine pudendal arteries was inhibited by an H_1 receptor blocker (Jakobsen et al., 1994). If the histamine response network behaves in the vasoactive milieu of the mammary glands in the same way it did in isolated arteries in vitro, then the antagonist of the H_1 receptor should have relaxed resistance to flow, just as blockers of the adenosine and NO responses affect mammary plasma flow (Lacasse et al., 1996; Madsen et al., 2001). However, we observed no effect of CPA on mammary plasma flow.

An issue that needs to be addressed in interpretation of our plasma flow results is that the measured plasma flow was not directed exclusively to the mammary glands but included femoral arterial flow to the hind limb. An effect of CPA on plasma flow to the hind limb could, therefore, either exaggerate or mask effects on mammary plasma flow. As with other arteries, both dilation and constriction of the cutaneous and femoral arteries have been observed, mediated by H_1 receptors (Clough et al., 1998; Arai and Chiba, 1999), so there is no consensus on how blood flow to the hind limb might be affected by CPA infusion. However, as discussed by Cant et al. (2002) and in agreement with measurement in lactating goats of blood flow simultaneously in the external iliac and external pudendal arteries (Bequette et al., 2001), mammary blood flow accounts for at least 80% of the iliac flow. This means that even a relatively large change in flow to the hind limb will have only minor effects on the iliac flow. Thus, our conclusion is that H_1 receptors in the mammary gland are not involved in bovine mammary blood flow regulation in vivo.

Role of Histamine in Milk Secretion

Although H_1 receptors did not influence mammary blood flow, there was a local effect of histamine mediated by H_1 receptors on milk yield. Yield from the infused udder half was negatively affected by infusion of CPA, whereas no effect was seen on milk yield from the noninfused udder half (data not shown). It has been suggested that histamine plays a role in milk ejection through effects on smooth muscle contraction (Eriksson et al., 1999). If this effect is mediated by H_1 receptors, it would explain the negative effect of CPA on milk yield. Milk fat content decreased by 16% when milk ejection was inhibited by continuous epinephrine infusion into goats (Leenanuruksa and McDowell, 1985) but we observed no effect on percentages of protein, fat, or lactose in milk.

The observation that feed intake dropped when CPA was infused indicates that CPA not only acted locally but also had a systemic effect. Intraperitoneal injection of the H_1 blocker dexbrompheniramine into goats at 2 mg/kg of $BW^{0.75}$ did not affect feed intake (Rossi et al., 1998). Our arterial infusion over 10 h amounted to a 6-fold higher dose of 12 mg/kg of $BW^{0.75}$ chlorpheniramine. Histamine in several species affects gastrointestinal blood flow by a vasodilatory effect mediated by H_1 receptors (Buckley et al., 1988; Rydning et al., 2001). It is therefore possible that blockade of the H_1 receptors over 10 h compromised gastrointestinal blood flow and thereby affected digestion and feed intake negatively. A direct effect on appetite centers in the brain does not seem likely, because histamine actually depresses feed intake and blockade of the H_1 receptors attenuates this effect (Masaki et al., 2001).

The decrease in arterial concentration of acetate when CPA was infused was likely a consequence of the putative CPA effect on the gastrointestinal tract because endogenous production of acetate is relatively low compared with the exogenous supply in ruminants (Pethick et al., 1981). The 25% lower acetate concentration resulted in a 23% decrease in acetate uptake by the mammary glands, but no change in milk output of its product, fat, relative to the other products, protein and lactose.

There was also a decrease ($P = 0.06$) in the $k \cdot Vol_{cap} \cdot N_{cap}$ product for TAG with CPA infusion that accounted for a decrease ($P = 0.09$) in $k \cdot Vol_{cap} \cdot N_{cap}$ for long-chain fatty acids. Because iliac plasma flow was not affected, the volume of perfused capillaries, $Vol_{cap} \cdot N_{cap}$, can be assumed to have remained constant between treatments, meaning that a drop in efficiency, k, of TAG uptake occurred. Disappearance of TAG between artery and vein is due to activity of endothelial lipoprotein lipase. We are unaware of a connection between the H_1 receptor and lipoprotein lipase activity, but there is a connection between histamine and heparin that may be significant. Heparin is stored in and released from mast cells, which also happen to be the major site of histamine storage in the body and the mammary glands (Maslinski et al., 1993). Heparin in the vascular lumen knocks mature lipoprotein lipase off the endothelium to severely decrease arteriovenous TAG hydrolysis (Augustus et al., 2003). Antagonism of the H_1 receptor may have interfered with the control of heparin release from mast cells and caused disengagement of lipoprotein lipase from the mammary endothelium. Although efficiency of TAG hydrolysis may have been reduced by CPA, net uptake of long-chain fatty acids across the mammary glands was not significantly affected.

Finding that CPA decreased feed intake and circulating concentrations of a milk precursor (acetate) raises the possibility that the reduced nutrient supply may have elicited a decrease in the rate of synthesis of milk components. A lower milk synthesis rate would be expected to decrease mammary blood flow (Linzell, 1974; Nielsen et al., 1990), countervailing the increase in mammary blood flow expected from the working hypothesis behind this experiment. However, the decline in milk yield in cows severely restricted in DMI did not commence until after 18 h of restriction (Toerien and Cant, 2007), and complete starvation of goats only caused a 10% decrease in milk yield after 8 h (Linzell, 1967). Thus, the likelihood of a nutrition-mediated decrease in mammary blood flow in this experiment, masking a CPA-mediated increase in flow, is negligible.

Role of AA in Mammary Blood Flow

It has been suggested that a deficient exogenous supply of individual AA may influence the rate of mammary blood flow in ruminants (Guinard and Rulquin, 1995). One of the AA that has been identified as potentially deficient for milk production is His (Vanhatalo et al., 1999). In the present study, cows were fed a low-protein diet and supplied with 44 g/h of exogenous AA, equivalent to approximately one-third of the total metabolizable AA supply, with or without His. Although a temporary His deficiency was apparent in the milk protein depression typical of the short-term infusion protocol, and in the increase in plasma AAN concentration, iliac plasma flow did not change. This finding lies in contrast to the 33% increase in mammary blood flow observed in goats subjected to a deficiency of approximately 45% of the metabolizable His supply for 6 d (Bequette et al., 2000). The hyperemia may require a few days to develop because of responses to the His deficiency that occur in extramammary organs of the cow. Although we have previously shown, with a simulation model of ATP balance in the milk-synthesizing system (Cant et al., 2003), that changes in circulating concentrations of energy metabolites due to extramammary effects can account for mammary hyperemias observed in response to both Met (Guinard and Rulquin, 1995) and His (Bequette et al., 2000) supply, no rigorous test of that hypothesis has been conducted. The mechanism by which an amino acid deficiency influences mammary blood flow does not involve histamine signaling through the H_1 receptor and remains unidentified.

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