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Mammary blood flow and metabolic activity are linked by a feedback mechanism involving nitric oxide synthesis

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ABSTRACT

To test which, if any, of the major milk precursors can elicit a rapid change in the rate of mammary blood flow (MBF) and to define the time course and magnitude of such changes, 4 lactating cows were infused with glucose, amino acids, or triacylglycerol into the external iliac artery feeding one udder half while iliac plasma flow (IPF) was monitored continuously by dye dilution. Adenosine and saline were infused as positive and negative controls, respectively, and insulin was infused to characterize the response to a centrally produced anabolic hormone. To test the roles of cyclooxygenase, NO synthase and ATP-sensitive K (K_{ATP}) channels in nutrient-mediated changes in blood flow, their respective inhibitors—indomethacin, N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), and glibenclamide—were infused simultaneously with glucose. Each day, 1 infusate was given twice to each cow, over a 20-min period each time, separated by a 20-min washout period. In addition, each treatment protocol was administered on 2 separate days. A 73% increase in IPF during adenosine infusion showed that the mammary vasodilatory response was quadratic in time, with most changes occurring in the first 5 min. Glucose infusion decreased IPF by 9% in a quadratic manner, most rapidly in the first 5 min, indicating that a feedback mechanism of local blood flow control, likely through adenosine release, was operative in the mammary vasculature. Amino acid infusion increased IPF 9% in a linear manner, suggesting that mammary ATP utilization was stimulated more than ATP production. This could reflect a stimulation of protein synthesis. Triacylglycerol only tended to decrease IPF and insulin did not affect IPF. A lack of IPF response to glibenclamide indicates that K_{ATP} channels are not involved in MBF regulation. Indomethacin and L-NAME both depressed IPF. In the presence of indomethacin, glucose infusion caused a quadratic 9% increase in IPF. Indomethacin is an inhibitor of mitochondrial function, so the glucose-induced increase in IPF was interpreted as feedback on mammary adenosine release from an anabolic response to glucose. Because NO synthase was not inhibited during indomethacin infusion, the feedback system is postulated to act through endothelial NO synthase. In the presence of L-NAME, glucose infusion had no effect on IPF, indicating that endothelial cyclooxygenase is not involved in glucose-induced changes in MBF.
Key words: mammary blood flow, nitric oxide synthase, cyclooxygenase, glucose

INTRODUCTION

Several tissues, including skeletal muscle (Jones and Berne, 1964), kidney (Haddy et al., 1958), heart (Scott et al., 1965), and the mammary glands (Linzell, 1974), have the ability to locally regulate their own blood flow according to metabolic activity in the tissue. A combination of feedforward and feedback mechanisms has been postulated to link metabolic activity with blood flow in skeletal and cardiac muscle. In feedforward mechanisms, products of energy metabolism stimulate blood flow, and in feedback mechanisms, they inhibit blood flow. One example of a feedforward mechanism involves production of CO_2 in muscle, which results in H^+-mediated activation of ATP-sensitive K ($\mathbf{K_{ATP}}$) channels in smooth muscle cells surrounding capillaries, causing relaxation and vasodilation (Deussen et al., 2012), presumably to improve nutrient supply for an elevated workload. Endothelial synthesis of the vasodilator NO also appears to be involved in CO_2-mediated hyperemia (Gurevicius et al., 1995). Another feedforward mechanism involves production of H_2O_2 during oxidative metabolism, which acts through voltage-gated K ($\mathbf{K_V}$) channels on smooth muscle cells (Rogers et al., 2006) and cyclooxygenase (**COX**)-mediated prostacyclin ($\mathbf{PGI_2}$) synthesis in endothelial cells (Thengchaisri and Kuo, 2003) to dilate capillaries. Adenosine is a

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feedback vasodilator that is released when intracellular ATP is low; it relaxes smooth muscle by activating K_{ATP} channels and endothelial NO synthase (Hein and Kuo, 1999; Deussen et al., 2012).

Adenosine, NO synthase, and PGI_2 have all been shown to be active in the mammary vasculature of lactating ruminants (Oguro et al., 1982; Nielsen et al., 1995a; Lacasse et al., 1996; Prosser et al., 1996), leading to the proposition that the mammary gland has the ability to locally control its own blood flow and, hence, arterial nutrient supply. A corollary to this proposition is that arterial nutrient supply should, in turn, have an influence on mammary blood flow (**MBF**) and, indeed, infusion of 90 g/h glucose into the external iliac artery supplying one udder half for 10 h reduced MBF by 16% (Cant et al., 2002), removal of histidine from an AA infusion into the abomasum caused an increase in MBF (Bequette et al., 2000), and elevation of plasma triacylglycerol (**TAG**) by fat supplementation of the diet caused a reduction in MBF:milk yield ratio (Cant et al., 1993). Accordingly, Cant and McBride (1995) and Cherepanov et al. (2000) constructed mathematical models of nutrient utilization in the mammary glands in which MBF is modified locally to match energy supply with expenditure. Mechanisms by which metabolic activity influences MBF were not specified because they have not been identified. The mammary models simulate changes in MBF that commence immediately on perturbation of arterial nutrient concentrations but we are unaware of any experimental data that demonstrate short-term responses of MBF to nutrient supply. Cherepanov et al. (2000) point out that the response delay would encompass rate of vasodilator production, reactivity of the capillaries, and hydraulic characteristics of the microvasculature. Blood flow to several organs, including the mammary glands, increases within seconds of administration of vasodilators into the arterial supply (Oguro et al., 1982; Rådegran and Hellsten, 2000). In muscle, blood flow reaches 50% of its maximum increase within 2 to 9 s of the onset of exercise (Rådegran and Saltin, 1998).

A greater understanding of the mechanisms involved in local regulation of mammary blood flow would allow predictive models to better account for variation in nutrient uptake and utilization for milk synthesis. To test which, if any, of the major milk precursors can elicit a rapid change in the rate of MBF, and to define the time course and magnitude of such changes, lactating cows were infused with glucose, AA, or TAG into the external iliac artery feeding one udder half while blood flow was monitored by dye dilution. Adenosine and saline were infused as positive and negative controls, respectively, and insulin was infused to characterize the response to a centrally produced, anabolic hormone. To test the roles of COX, NO synthase, and K_{ATP} channels in nutrient-mediated changes in blood flow, their respective inhibitors indomethacin, N_{ω}-nitro-L-arginine methyl ester hydrochloride (**L-NAME**), and glibenclamide were infused simultaneously with glucose.

MATERIALS AND METHODS

Animals

All animal procedures and holding facilities were approved by the Animal Care Committee of the University of Guelph. Four multiparous Holstein cows, at 580 ± 29.3 kg of BW and 54 ± 8.9 DIM (±SEM) were fitted with polyurethane catheters in both left and right subcutaneous abdominal veins (milk veins) and with polyethylene catheters in both iliac arteries, introduced through the saphenous arteries as described by Maas et al. (1995). The external iliac position was confirmed by injection of Evan's Blue dye into the arterial catheters (Cant et al., 2001).

After 2 d of recovery in box stalls, cows were housed in tie stalls with mattresses and wood shavings. They were milked and fed a corn silage-based TMR (50.5% DM, 17.7% CP, 32.6% NDF, 3.6% fat, 1.74 Mcal of NE_L/kg on a DM basis) twice daily at 0800 and 1800 h. Refusals were weighed at 0730 h daily to determine feed intake. Milk was sampled at each milking and analyzed by infrared spectroscopy for protein, fat, and lactose (AOAC International, 1996).

Infusion Protocols

Commencing on d 5 after surgery, cows were subjected twice daily to a 95-min infusion protocol into one external iliac catheter, starting at 1000 or 1400 h. *Para*-aminohippuric acid (**PAH**; Na salt) was infused continuously at 100 mg/min throughout the entire 95 min to estimate iliac plasma flow by arteriovenous dilution. As depicted in Figures 1, 2, 3, 4, 5, and 6, a treatment solution was infused for 20 min, commencing at 30 min of PAH infusion, and after a second baseline period, at 70 min of PAH infusion. The same treatment was infused both times. Treatments were 0.38 g/min of NaCl, 3 mg/min of adenosine (Sigma Chemical Co., St. Louis, MO), 2.2 g/min of glucose (Sigma Chemical Co.), 2.11 g/min of a complete AA mix (Table 1), 0.87 g/min of TAG (10% Liposyn; Abbott Laboratories Ltd., Montreal, Quebec, Canada), 20 μg/min of insulin (Sigma Chemical Co.), 2.0 mg/min of indomethacin (Sigma Chemical Co.), 25 mg/min of L-NAME (Sigma Chemical Co.), or 2.0 mg/min of glibenclamide (Sigma Chemical Co.). Glucose and AA solutions were prepared at 220 and 211 g/L, respectively. The complete