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## Nitric oxide and the control of mammary blood flow

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### ABSTRACT

Mammary blood flow (MBF) is a major determinant of the rate of substrate supply for milk synthesis, but the control mechanism(s) of MBF has not been elucidated. Our objective was to test the hypothesis that local production of the vasorelaxant nitric oxide (NO) can regulate MBF. Thus the response in MBF to intra-arterial infusion of the NO donor diethylamine NONOate (NONate), and the inhibitor of NO synthesis N<sup>ω</sup>-Nitro-arginine (NNA) was measured in four lactating Saanen goats. MBF was measured using a transit time ultrasonic flow probe implanted around the external pudic artery of one gland and infusions were made via a catheter inserted into the same artery. In two goats, a flow probe was implanted on the pudic artery of the contralateral gland. NONate was infused for 60 min. at 1.67 and 16.7 µg/min and induced an increase ( $P < 0.01$ ) in MBF which averaged 190 and 243% of the preinfusion MBF, respectively, for the duration of the infusion. There was no significant difference between the doses. The effect was more pronounced in the infused gland compared with the non-infused gland (201 vs 111% of the preinfusion MBF;  $P < 0.05$ ) suggesting a direct effect on mammary vasculature. In contrast to NONate, NNA infused at 1 and 2.5 mg/min for 60 min decreased MBF by up to 35% ( $P < 0.05$ ) and the effect persisted for more than 90 minutes after infusion. Infusion of 4 mg/min of arginine the NO precursor, with NNA markedly reduced the latter's ability to decrease MBF ( $P < 0.05$ ). Again the effect was more pronounced in the infused compared with the non-infused gland ( $P < 0.05$ ). Together, these results indicate that NO is a major vasorelaxant of the mammary gland vasculature and that this substance is produced within the gland.

**Keywords:** blood flow; nitric oxide; mammary.

### INTRODUCTION

Mammary blood flow (MBF) is a major determinant of the rate of substrate supply for milk synthesis (Davis and Collier, 1985). Therefore, it is not surprising that there is a close correlation between mammary blood flow and milk production (Linzell, 1974). It is well established that mammary blood flow is affected by several factors such as physiological state (Linzell, 1974), level of milk production (Linzell, 1974), frequency of milk removal (Stelwagen *et al.*, 1994), mammary infection (Dhondt *et al.*, 1977) and fasting (Chaiyabutr *et al.*, 1980).

The nature of the control mechanism of mammary blood flow has not been fully elucidated. Reduction of mammary blood flow occurred during fasting even when the gland had been denervated (Chaiyabutr *et al.*, 1980) implying that vasoconstriction is achieved by local or humoral factors. Recently, it has been discovered that vascular endothelial cells, and some other cell types, can synthesise the inorganic free-radical gas nitric oxide (NO) which is a vasorelaxant for several blood vessels (Knowles and Moncada, 1992). Nitric oxide is synthesised from L-arginine (Palmer *et al.*, 1988) and relaxes vascular smooth musculature via the stimulation of guanylate cyclase (Furghott and Vanhoutte, 1989). The synthesis of NO can be inhibited by several L-arginine analogues, and this inhibition increases resistance in vascular beds (Knowles and Moncada, 1992). However, it is not known if NO has any effect on mammary gland vasculature.

The purpose of this experiment was to investigate whether NO can affect MBF and if this substance is produced within the gland.

### MATERIALS AND METHODS

In four lactating Saanen goats, the external pudic artery supplying one gland was cannulated with polyvinyl tubing (1.0 mm OD, 0.5 mm ID) according to the method described by Fleet and Mephan (1983). At the same time, a transit-time ultrasonic flow probe (Transonics Inc, Ithaca, NY) was implanted around the artery, upstream of the catheter. In two goats, the external pudic artery of the contralateral gland was also implanted with a flow probe. The animals were allowed to recover from surgery for at least a week before commencing infusion.

The first experiment was conducted to examine if NO can affect MBF. The NO donor, Diethylamine NONOate (NONate; Cayman Chemical Co., Ann Arbor, MI) was dissolved in 50 mM Na phosphate buffer (pH 9) containing 0.15 M NaCl (PBS) to give 0, 5 and 50 µg/ml and infused via the pudic artery catheter of the four goats for 1 hour at 20 ml/hour. Mammary blood flow was recorded continuously from 30 minutes before to 60 minutes after the infusion.

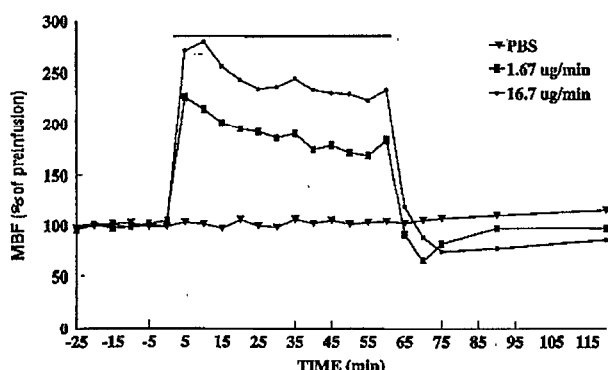
In the second experiment, the effect of infusion of the NO synthase inhibitor N<sup>ω</sup>-Nitro arginine (NNA) on MBF was investigated. Saline, 1 and 2.5 mg NNA/min and 1 mg NNA and 4 mg arginine/min were infused for 60 minutes at 20 ml/hour via the pudic artery catheter of the four goats. Mammary blood flow was recorded continuously from 15 minutes before to 180 minutes after the infusion.

Each data point represents the average of 10 minutes of recording. These values were transformed as a percentage of the preinfusion period and analysed using the repeated measurement procedure of GLM (SAS, 1985).

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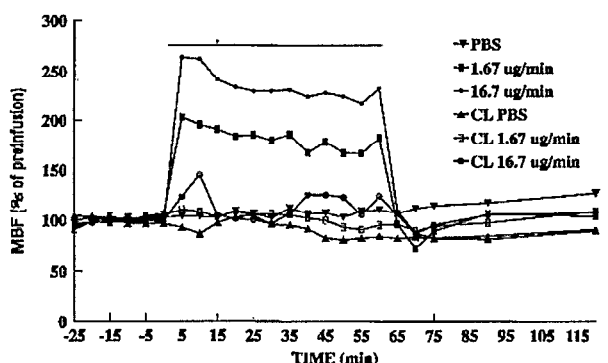
## RESULTS

**FIGURE 1:** Mammary blood flow (MBF) response to 60 minutes infusion of PBS, 1.67 and 16.7  $\mu\text{g}/\text{min}$  of NONate in four lactating goats. The increase in MBF during NONate infusion was significant ( $P<0.01$ ). Solid bar indicates period of infusion.



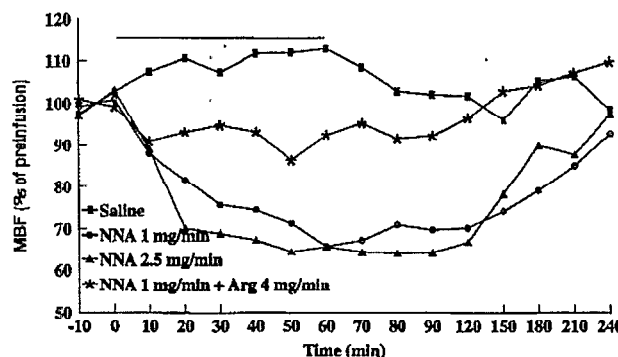
Mammary blood flow response to NONate and PBS infusion are depicted in fig. 1. PBS infusion had no effect while both doses of NONate induced a sharp increase in MBF. While the MBF response declined slightly during infusion of NONate, MBF was still significantly elevated at the end of the infusion period ( $P<0.05$ ). The response to the two doses was not different. For the whole infusion period, MBF averaged 102, 190 and 243 % of the preinfusion MBF, for the 0, 1.67 and 16.7  $\mu\text{g}/\text{min}$  infusions respectively. Once the infusion of NONate was stopped, MBF dropped below preinfusion values and remained depressed ( $P<0.05$ ) up to 60 min postinfusion. Blood flow in the contralateral gland was not significantly altered ( $P>0.25$ ; Fig. 2) and averaged 96, 104 and 117 % of the preinfusion MBF, for the 0, 1.67 and 16.7  $\mu\text{g}/\text{min}$  infusions respectively.

**FIGURE 2:** Infused and non-infused (CL) mammary gland blood flow (MBF) response to 60 minutes infusion of PBS, 1.67 and 16.7  $\mu\text{g}/\text{min}$  of NONate in two lactating goats. The increase in MBF during NONate infusion was significant ( $P<0.05$ ) for the infused gland only. Solid bar indicates period of infusion.

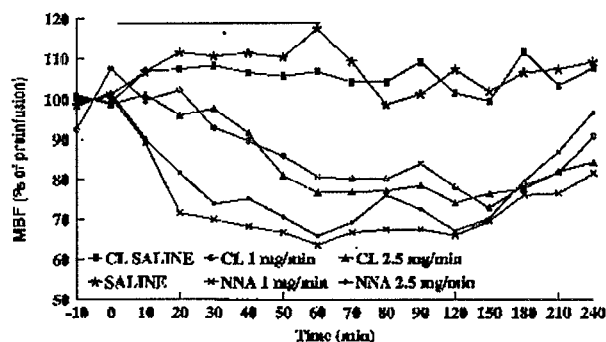


Infusion of 1 and 2.5 mg/min of NNA reduced MBF down to 65% of the preinfusion level ( $P<0.01$ ; Fig. 3). The depressive effect of NNA persisted for more than 90 min after the end of the infusion. Infusion of 4 mg/min of arginine, the physiological NO precursor, with NNA markedly reduced the latter's ability to decrease MBF ( $P<0.05$ ). NNA infusion also reduced MBF in the contralateral gland ( $P<0.05$ ; Fig. 4)

**FIGURE 3:** Mammary blood flow (MBF) response to 60 minutes infusion of saline, 1 and 2.5 mg/min of NNA and 1 mg of NNA + 4 mg of arginine/min in four lactating goats. The decrease in MBF during NNA infusion was significant ( $P<0.01$ ). Solid bar indicates period of infusion.



**FIGURE 4:** Infused and non-infused (CL) mammary gland blood flow (MBF) response to 60 minutes infusion of saline, 1 and 2.5 mg/min of NNA and 1 mg of NNA + 4 mg of arginine/min in four lactating goats. The decrease in MBF during NNA infusion appeared earlier in the infused gland ( $P<0.05$ ). Solid bar indicates period of infusion.



but the reduction was more pronounced and occurred earlier in the infused compared with the non-infused gland ( $P<0.05$ ).

## DISCUSSION

The present study demonstrates a rapid increase in MBF in response to intramammary infusion of the NO donor diethylamine NONOate. As NO has a very short half life (~5 seconds), it had to be supplied by a NO donor. Diethylamine NONOate dissociates in aqueous solution to give the free amine and NO. The reaction is pH dependent. It is very slow at pH 8.5 but rapid ( $t_{1/2} = 2.1$  minutes) at pH 7.4. To the knowledge of the authors, this is the first time that the vasorelaxant activity of NO has been tested on mammary gland vasculature. The increase in MBF was limited to the infused gland only. Therefore, this increase was most likely the result of a local reduction in resistance to blood flow.

Intramammary infusion of NNA resulted in a dramatic reduction of MBF. NNA, an L-arginine analogue, is a potent inhibitor of NO synthase and hence local NO production (Ishii, *et al.*, 1990). In the present study, the effect of NNA was markedly reduced by co-infusion of arginine, the usual substrate of NO synthase. Fleet *et al.*, (1993) reported that another L-arginine analogue, N<sup>G</sup> methylarginine, substantially reduced the stimulating effect of oxytocin on MBF in lactating goats. However, they did not report the effect on basal blood flow.

Even though infusion of NNA reduced blood flow of both the infused and the non-infused glands, the earlier response in the infused gland is indicative of a local action. It could be argued that a part of the depressing effect of NNA on MBF could be the result of a lower cardiac output. Tresham *et al.*, (1991) reported a small decrease in heart rate after an injection of NNA to sheep. However, they also observed a 20% increase in arterial blood pressure; which means that if local resistance of an organ is not affected by NNA, its blood flow should increase. It is much more likely that the effect of NNA on the contralateral gland's vasculature is due to the gradual accumulation of NNA in the general circulation. The persistency of the MBF depression during the postinfusion period is in agreement with this hypothesis.

As NO is very unstable, it needs to be produced locally to efficiently induce a vasorelaxation. Endothelial cells lining blood vessels of several different tissues secrete NO (Palmer *et al.*, 1988) and removal of endothelium inhibited the relaxing effect of acetylcholine on isolated artery (Furchgott and Zawadzki, 1980). This endothelium-derived relaxing factor was subsequently identified as NO (Furchgott and Vanhoutte, 1989). In addition to endothelial cells, several other cell types possess NO synthase, including kidney epithelial cells (Tracey *et al.*, 1994). This suggests mammary production of NO might not be limited to the endothelium.

In conclusion, these results indicate that NO is a potent vasorelaxant of the mammary gland vasculature and that this substance is produced within the gland; thus, suggesting that it plays an important role in the local control of mammary gland blood flow.

#### ACKNOWLEDGMENTS

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#### REFERENCES

- Chaiyabutr, N., Faulkner, A., Peaker, M. 1980. Effect of starvation on the cardiovascular system, water balance and milk secretion in lactating goats. *Research in Veterinary Science* **28**: 291-295
- Davis, S.R., Collier, R.J. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. *Journal of Dairy Science* **68**:1041-1058
- Dhondt, G., Burvenich, C. Peeters, G. 1977. Mammary blood flow during experimental *Escherichia coli* endotoxin induced mastitis in goats and cows. *Journal of Dairy Research* **44**:433-440
- Fleet, I.R., Davis, A.J., Richardson, M., Heap, R.B. 1993. The stimulation of mammary blood flow by oxytocin and the potential role of locally released nitric oxide in the goat. *Journal of Reproduction and Fertility* **198** (abstract)
- Fleet, I.R., Mepharm, T.B. 1983. Physiological methods used in the study of mammary gland substrate utilisation in ruminants. *In: Biochemistry of Lactation*. Mepharm, T.B. (ed.) Elsevier, Amsterdam, p. 469-492
- Furchgott, R.F., Zawadzki, J.V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle in acetylcholine. *Nature* **288**: 373-376
- Furchgott, R.F., Vanhoutte, P.M. 1989. Endothelium-derived relaxing and contracting factors. *FASEB Journal* **3**: 2007-2018
- Ishii, K., Kerwin, J.R. Jr., Murad, F. 1990. N<sup>ω</sup>-Nitro-L-arginine: a potent inhibitor of the L-arginine-dependent soluble guanylate cyclase activation pathway in LLC-PK<sub>1</sub> cells. *Canadian Journal of Physiology and Pharmacology* **68**: 749-751
- Knowles, R.G., Moncada, S. 1992. Nitric oxide as a signal in blood vessels. *TIBS* **17**: 399-402
- Linzell, J.L. Mammary blood flow and methods of identifying and measuring precursors of milk. Page 143 in *Lactation*, Vol 1., B.L. Larson, V.R. Smith ed. Academic Press, New York, NY
- Palmer, R.M., Ashton, D.S., Moncada, S. 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**: 664-666
- Statistical Analysis System Institute Inc. 1985. *SAS User's Guide*. Statistics, Version 5. Edition SAS Institute Inc. Cary, NC 956 pp
- Stelwagen, K., Davis, S.R., Farr, V.C., Prosser, C.G. Sherlock, R.A. 1994. Mammary epithelial cell tight junction integrity and mammary blood flow during an extended milking interval in goats. *Journal of Dairy Science* **77**: 426-432
- Tracey, W.R., Pollock, J.S., Murad, F., Nadane, M., Forstermann, U. 1994. Identification of an endothelial-like type III NO synthase in LLC-PK<sub>1</sub> kidney epithelial cells. *American Journal of Physiology* **266**: 22-28
- Tresham, J.J., Dusting, G.J., Coghlan, J.P., Witworth, J.A. 1991. Haemodynamic and hormonal effects of N-Nitro-L-arginine, an inhibitor of nitric oxide biosynthesis, in sheep. *Clinical and Experimental Pharmacology and Physiology* **18**: 327-330