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Vasodilatory properties of parathyroid hormone-related protein in the mammary gland of the lactating goat

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ABSTRACT

Human synthetic parathyroid hormone-related protein (PTHrP) increased mammary blood flow (MBF) following close-arterial infusion via the external pudic artery of lactating goats. MBF increased 74±8% within 30 min of the start of continuous infusion of PTHrP compared with 10±3% from control. However, by 90 min MBF decreased and was not different from control for the remainder of the infusion. Elevation in plasma concentrations of calcium and decrease in phosphate during PTHrP suggests this was not due to altered activity of PTHrP, but may relate to down-regulation of response or production of counter-regulatory vasoconstrictive agents within the gland. However, this problem can be alleviated when PTHrP is infused in a pulsatile fashion. Thus, PTHrP may be usefully employed in studies designed to raise MBF during lactation.

Keywords: PTHrP, mammary blood flow, goats.

INTRODUCTION

Parathyroid hormone-related protein (PTHrP) was first identified as the factor responsible for humoral hypercalcemia of malignancy and has been isolated from tumors (Martin et al., 1989). PTHrP is also expressed by a variety of normal tissues including the lactating mammary gland (Thiede and Rodan, 1988; Selvanayagam et al., 1991) and is present in milk at high concentrations (Budayr et al., 1989; Ratcliffe et al., 1990; Thurston et al., 1990). The small, but positive correlation found between concentrations of PTHrP in bovine milk and total calcium (Law et al., 1991) and the increased secretion of calcium, magnesium and phosphate into milk following intravenous infusion of PTHrP (Barlet et al., 1992) has led to the suggestion that the role of this protein in the mammary gland is local regulation of calcium transport from blood to milk. However, more recent data from the rat shows a lack of correlation between milk calcium and PTHrP, therefore questioning such a role for this protein (Yamamoto et al., 1992).

In addition to its possible influence on calcium secretion in the mammary gland, PTHrP has been shown to be vasoactive in the lung (Goharderakhsh and et al., 1992) and kidney (Wuqast, Baslkin and Vlasuk, 1987), therefore raising the possibility of a role for PTHrP as a local regulator of mammary blood flow. This suggestion is further strengthened by the observation that the rise in mammary blood flow at lactogenesis in goats is preceded by an increase in PTHrP output into the mammary venous effluent (Ratcliffe, Thompson, Care and Peaker, 1992). The lactating mammary gland is critically dependent upon an adequate supply of nutrients and hormonal stimuli from blood to sustain optimal milk synthesis so the importance of factors regulating supply of blood to the mammary gland is obvious. Thus this study was initiated to examine the vasoactive properties of PTHrP in the mammary gland of the goat.

MATERIALS AND METHODS

Three lactating Saanen goats, yielding 2.0-2.5 kg of milk daily, were housed indoors. The external pudic artery supplying one gland was cannulated with polyvinyl tubing (0.8mm OD, 0.5mm ID) according to the method described by Fleet and Mepham (1983). At the same time a transit-time ultrasonic flow probe (Transonics Inc., Ithaca, NY) was implanted around the artery upstream of the catheter. The animals were allowed to recover from the surgery for at least a week before commencing infusions.

Three experiments were conducted to examine the vasoactive properties of synthetic human PTHrP 1-34 (Peninsula Laboratories Inc, Belmont, Ca). In the first, PTHrP was infused for 6 h at 366 nmol/l (19ml/h) into three goats. The material was dissolved in 0.9% NaCl containing 0.01% BSA and control infusions were with vehicle alone. On the morning of the infusion, the goats were milked at 0730 h by machine then hand milked at 0800, 0900, 1000 and thereafter at 2 hourly intervals until 2200 h. Each milking was preceded by intravenous injection of 200 mU oxytocin. Infusions began at 1000 h and MBF was measured for a total of 5 min every 30 min commencing 0800 h. The average flow over this 5 min period was calculated and recorded. Blood (8 ml) from the jugular vein was collected into EDTA hourly beginning 0800 h and plasma prepared by centrifugation. Concentrations of calcium and phosphate in plasma were measured by atomic absorption spectrophotometry and colorimetric methods, respectively. In the second experiment, doses of PTHrP (0, 73.2, 122, 244, 366, 488 nmol/l; 19ml/h) were infused via the pudic arterial catheter of two goats for 15 min with mammary blood flow (MBF) recorded continuously. Again the material was dissolved in 0.9% NaCl containing 0.01% BSA and responses were compared with MBF during infusion of vehicle alone.

In a further experiment, PTHrP was infused at 366 nmol/
RESULTS

The MBF response to a 6 h continuous infusion of PTHrP into three goats is shown in Fig 1. By 30 min of the start of infusion of PTHrP MBF increased 74±8% compared with the average rate of flow for the two hours preceding the infusion and 10±3% during infusion of vehicle alone. However, by 90 min infusion of PTHrP MBF had decreased to be no different from control infusions. This level of flow was maintained until approximately 4 h when, in both control and PTHrP infusions, MBF increased. Once infusions stopped MBF decreased 27% relative to control treatment, but increased again soon after to be 46% above control by 90 min after the end of infusion.

FIGURE 1: Change in blood flow through the infused gland of three lactating goats during infusion of PTHrP (●) or saline (○). Infusion was for 0-6 h and mammary blood flow (MBF) was expressed as a percentage of average rate of flow for the two hours preceding start of infusion. Values are mean±SEM.

Milk yield from the infused gland was not significantly different from the non-infused gland (data not shown). Therefore yields have been combined and, to reduce inter-animal variation, these have been expressed as a ratio of the calculated hourly yield obtained on the day before infusion. These data are presented in Fig 2. There was no significant difference between control or PTHrP infusions in terms of milk yield at any time. Plasma concentrations of calcium increased non-significantly from 2.2±0.1 before to 2.5±0.2 mM after 6 h infusion of PTHrP. Phosphate concentrations declined from 2.4±0.2 mM to 1.4±0.4 mM (P<0.01). In contrast, neither calcium or phosphate levels were altered by infusion of vehicle alone.

The effect of infusion of various doses of human synthetic PTHrP 1-34 on MBF in the lactating goat is given in Fig 3. Since the basal rate of blood flow varied considerably between the two goats (averaging 130 and 570 ml/min) the doses infused are given as the concentration in the blood supplying the gland, calculated by dividing the infusion rate in nmole/min by the average blood flow in ml/min obtained over a 5 min period prior to infusion. This rate of flow was essentially the same for the same animal over the course of the experiment. The response is expressed as the percentage change in MBF compared with 15 min infusion of vehicle alone. Data from the two goats were combined to yield the complete dose response curve. The response curve did not plateau over the range of concentrations of PTHrP used.

FIGURE 3: Increase in mammary blood flow (MBF) in response to varying doses of PTHrP infused into two goats (●,○). The doses infused are given as the concentration in the blood supplying the gland, calculated by dividing the infusion rate in nmole/min by the average blood flow in ml/min obtained over a 5 min period prior to infusion. The response is expressed as the percentage change in MBF compared with 15 min infusion of vehicle alone.

In the third experiment, where PTHrP was infused in a pulsatile fashion every 15 min over 3 h, MBF increased from 200 to 400 ml/min for one goat and 700 to 900 ml/min for the other with each pulse of PTHrP, but declined immediately after each pulse (Fig 4). By the beginning of the next pulse of PTHrP MBF had returned to baseline. The percentage response to each pulse was similar even though basal MBF tended to decline as time progressed.
FIGURE 4: Mammary blood flow (MBF) before, during and after a series of 15 min infusions of PTHrP given every 30 min for 3 h commencing at 0 h. The beginning of each pulse of PTHrP is indicated by the arrows. Results are data from two different goats.

DISCUSSION

The present study demonstrates a rapid increase in MBF in the gland of the lactating goat in response to intramammary infusion of human synthetic PTHrP (1-34). The effect of a continuous infusion of PTHrP for 6 h, however, was only transient. In view of this it was not surprising to see no difference between control and PTHrP infusion in terms of the rate of milk secretion. The increase in calcium and decrease in phosphate concentrations in plasma during infusion of PTHrP compared with vehicle only is consistent with the known systemic effects of PTHrP (Barlet et al., 1992), indicating the biological activity of the infused material was not compromised in vivo.

One interpretation of the transient response to continuous infusion of PTHrP is local production of vasoconstrictive agents or reduced production of vasodilatory agents within the gland. Alternatively, since the biological actions of PTHrP are mediated by binding to the PTH receptor (Martin et al., 1989), down-regulation of these receptors within the mammary tissue could also be the cause. While the former mechanism can readily account for the initial decrease and subsequent increase in MBF once exogenous PTHrP was withdrawn, the latter would only be consistent with this phenomena if endogenous PTHrP played a significant role in local regulation of MBF.

The relative importance of endogenous PTHrP in regulating MBF is not known. The lowest dose of PTHrP infused in the present study gave a 17% response, which is only just discernable above a background variation of 10% in natural flow. This dose was equivalent to a concentration of 4 pmol/l in blood supplying the mammary gland, whereas concentrations of endogenous PTHrP in mammary venous plasma of goats 7 days after parturition average only 1.2 pmol/l (Ratcliffe et al., 1992). Therefore the doses of PTHrP infused during the present study must represent pharmacological levels.

In summary, the present data suggest exogenous PTHrP is not a potent stimulator of MBF, at least when administered intra-arterially. Nevertheless, exogenous PTHrP may be of value in studies determining the relationship between MBF and milk yield for instance. In this regard, however, preliminary experiments suggest pulsatile infusion would maintain high rates of blood flow more successfully than a continuous infusion. While the data also suggest endogenous PTHrP may not be an important regulatory component of blood flow in the mammary gland of the lactating goat no definitive conclusion can be made until local tissue concentrations of PTHrP are known.

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