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Artificial Induction of Lactation in Ewes: The Relative Importance of Oxytocin and the Milking Stimulus

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Abstract

Eight ovariectomized and four intact ewes were given oestrogen plus progesterone to develop the mammary glands. The intact ewes (group A) and four ovariectomized ewes (group B) then received four injections each day of 1 i.u. syntocinon for 5 days whereas the other four ovariectomized ewes (group C) received placebo injections of 0·9% saline. Milking commenced the day after the last of these injections.

Yields of mammary secretion on the first day of milking—representing secretion accumulated during the injection of syntocinon or saline—for ewes in groups A and B were significantly higher than yields from group C (control) ewes. After 12 days of milking, yields of secretion from group A ewes were significantly higher than yields from group B ewes which in turn were significantly higher than yields from ewes in group C.

Introduction

It was observed during the course of recent experiments that simply milking non-pregnant ewes with developed mammary glands initiated secretion of small volumes of fluid similar in composition to normal ovine milk (Fulkerson and McDowell 1974*a*). This finding suggested that the milking stimulus *per se* or hormones released by it may act as lactogenic triggers in sheep. In this regard, Delouis and Denamur (1967) reported that milk secretion could be initiated in late pregnant ewes following intravenous injection of 1 i.u. syntocinon (synthetic oxytocin) four times a day. Moreover, in preliminary experiments at this laboratory it was found that injections of syntocinon initiated copious milk secretion in intact ewes previously given oestrogen plus progesterone to develop mammary glands (Fulkerson, unpublished data).

The ewes in both of the above experiments were milked during the period syntocinon was injected so the involvement of the milking stimulus *per se* in the lactogenic response cannot be assessed. In the present studies attempts were made to determine whether syntocinon administered to non-pregnant intact and ovariectomized ewes with developed mammary glands would trigger milk secretion in the absence of the milking stimulus.

Materials and Methods

Sheep

Twelve nulliparous crossbred (Border Leicester × Merino) ewes were housed in an open shed lit by natural light and fed *ad libitum* a ration containing 90% lucerne chaff and 10% concentrate pellets. All ewes were free of obvious abnormalities of the mammary glands.

When the experiment commenced the intact ewes were in anoestrus and ewes from the same flock remained in anoestrus throughout the period of the experiment. Eight ewes were ovariectomized 3 weeks before the experiment commenced.

Hormones

Progesterone (Calbiochem, La Jolla, California) and oestradiol benzoate (Schering AG, Berlin) were dissolved in ethanol and then suspended in peanut oil. Syntocinon (Sandoz Ltd, Basle) was diluted in sterile 0·9% (w/v) saline at a final concentration of 0·5 i.u./ml.

Experimental Procedure

The mammary glands of each ewe were developed, over a period of 30 days, by injecting subcutaneously every third day 60 mg progesterone plus 240 μg oestradiol benzoate. Three days after the last of these injections ewes were treated for a further 5 days as follows:

Group A: four intact ewes—four intravenous injections each day (at 0800, 1200, 1600 and 2000 h) of 1 i.u. syntocinon;

Group B: four ovariectomized ewes—as four group A;

Group C: four ovariectomized ewes—four intravenous injections each day (at 0800, 1200, 1600 and 2000 h) of 2·0 ml saline.

Milking commenced on the day following the last injection of syntocinon or saline. Milk yields were recorded daily and samples of secretion were stored at −16°C pending analysis for lactose (Fulkerson and McDowell 1974*a*).

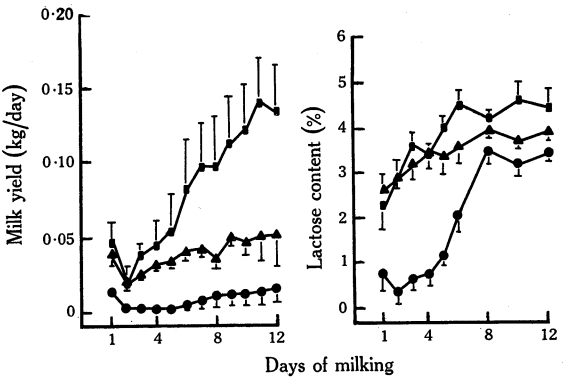

Fig. 1. Daily yield and lactose content of mammary secretion for the three groups of ewes commencing on the first day of milking. Values shown for day 1 represent cumulative production until that time. ■ Group A. ▲ Group B. ● Group C. Plotted points represent mean values and standard errors are shown as vertical bars.

Results

The results are presented in Fig. 1. On the first day of milking, yields of secretion (representing cumulative production until that time) from ewes in groups A and B (ewes treated with syntocinon) were similar and significantly greater ($P < 0·05$) than yields for group C (control) ewes. After 12 days of milking, yields of secretion from group A ewes (0·130 kg/day) were significantly greater ($P < 0·05$) than yields from group B ewes (0·054 kg/day), which were in turn significantly greater ($P < 0·05$) than yields from group C ewes (0·016 kg/day).

Concentrations of lactose in secretion from ewes in groups A and B, on the first day of milking, were similar (*c.* 2·4%) and significantly higher ($P < 0·001$) than

concentrations from group C ewes (0·75%). By 5–6 days after milking commenced, concentrations of lactose in secretion from ewes in groups A and B had reached stable levels (*c.* 4·0%) similar to those found in normal ovine milk (Fulkerson and McDowell 1974*b*). On the other hand, concentrations of lactose in secretion from group C ewes continued to increase throughout the period of the experiment and after 12 days of milking concentrations had reached 3·50%.

Discussion

Results of previous attempts to demonstrate that oxytocin initiates milk secretion have been equivocal. Haun (1959) first reported that pitocin (an extract of oxytocin from the posterior pituitary) initiated milk secretion in non-pregnant rabbits with developed mammary glands. However, this finding was refuted by subsequent workers who were unable to demonstrate that pitocin was lactogenic in oestrogen-primed rats or rabbits (Meites 1959; Meites *et al.* 1960). More recently Delouis and Denamur (1967) reported that injection of syntocinon initiated secretion of milk in pregnant ewes. The results of the present studies extend the latter findings by showing that injections of syntocinon given to intact and ovariectomized ewes with developed mammary glands initiate secretion of substantial volumes of fluid resembling normal ovine milk.

Yields of secretion from the ewes in the present studies were considerably less than those reported earlier for ewes which had been induced to lactate after being given ovarian steroids for 60 days (Fulkerson and McDowell 1974*a*). In this earlier study milk yields of ewes induced to lactate artificially were about 85% of yields from similar ewes lactating after normal pregnancy. Results of recent experiments at this laboratory (Fulkerson, Gow and Darton, unpublished data) suggest that yields of the ewes in the present study would have been higher had glands been developed with ovarian steroids for longer than 30 days. Moreover, it has been found that the sheep used in the present studies were of a strain which is relatively low-yielding when lactating after normal pregnancy (Fulkerson, unpublished data).

It has been shown in the virgin goat that simply milking for prolonged periods initiates secretion of milk-like fluid (Cowie *et al.* 1968). The results of more recent studies at this laboratory showed that simply milking non-pregnant ewes with developed mammary glands led to secretion of small volumes of milk (Fulkerson and McDowell 1974*a*). These findings demonstrate that the milking stimulus is important for the initiation of lactation.

Clearly, it is difficult to separate any effect of milking from the effect of oxytocin on initiation of milk secretion. In spite of this it is considered that a successful attempt to do so was made in the present experiment. It was apparent that ewes treated with syntocinon (groups A and B) yielded significantly greater amounts of secretion on the first day of milking than the control (group C) ewes. Moreover, the levels of lactose in mammary secretion from ewes in groups A and B were significantly higher at this time than those for group C ewes. These differences were still apparent 12 days after milking commenced when the yield and lactose content of secretion from ewes treated with syntocinon were higher than those for control ewes.

It could be argued that the dose of oxytocin administered in the present experiment was excessive. Levels of oxytocin in peripheral blood immediately after injection would have been 5×10^{-4} i.u./ml—assuming a blood volume of *c.* 2 litres. This

level is within the range of values given by Cleverley (1968) and is only 2–3 times the levels reported by Momongan and Schmidt (1970) in cows during milking. Far higher values have been recorded during parturition in the sheep (Fitzpatrick and Walmsley 1964). These findings suggest that the dose of syntocinon administered to ewes in the present experiment would have resulted in physiological levels in peripheral circulation.

Although the results of the present experiments show that syntocinon is lactogenic in the primed ewe it is not possible to define the mechanism by which oxytocin exerts this lactogenic effect. In spite of this, the observation that yields of secretion from ovariectomized ewes were substantially lower than those of intact ewes raises the possibility that oxytocin may be lactogenic by virtue of an unknown interaction with the ovary. In this connection, it has been found that syntocinon is as effective as dexamethasone or high doses of oestrogen in initiating lactation in intact ewes with developed mammary glands (Fulkerson *et al.* 1975*b*). On the other hand, in ovariectomized ewes with developed glands, the lactogenic response to syntocinon has been found to be substantially less than that to dexamethasone or high doses of oestrogen (Fulkerson *et al.* 1975*a*).

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