Effect of exogenous oxytocin on the leakage of lactose from the mammary gland and on suckling-induced plasma prolactin in the lactating rat

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The leakage of lactose from the mammary gland into plasma and the increase in the suckling-induced concentration of prolactin in the plasma of oxytocin-injected lactating rats suckling different numbers of pups were investigated. Rats nursing eight pups were isolated from their litter for 6 h and injected i.v. with either 1 iu oxytocin or saline and allowed to resuckle either two, eight or 12 pups for 1 h. The concentration of lactose in plasma increased significantly in rats injected with oxytocin and allowed to resuckle two or eight pups; however, there was no difference in rats allowed to resuckle 12 pups. When compared within oxytocin-injected groups, the concentration of lactose was significantly lower in rats allowed to resuckle eight or 12 pups than in rats allowed to resuckle only two pups. Plasma prolactin concentrations increased during the suckling period in mothers suckling either eight or 12 pups, and oxytocin diminished the increase in prolactin concentration in both groups. In rats suckling two pups, prolactin release was not observed. The second objective of this study was to determine the relationship between the leakage of lactose from the mammary gland by oxytocin injection and suckling. Mothers that had the three left nipples of their mammary glands occluded were isolated from their litter of eight pups for 6 h and were allowed to resuckle with eight pups for 1 h. At the start of resuckling, 0.01, 0.1 or 1 iu oxytocin was injected i.v. The amount of lactose in the occluded mammary glands was not altered by the administration of either 0.01 or 0.1 iu of oxytocin or by saline, whereas 1 iu oxytocin significantly decreased it. The amount of lactose in the non-occluded side decreased with oxytocin in a dose-dependent manner. The present study indicates that the transfer of lactose from milk to plasma by exogenous oxytocin diminishes with milk removal, or that unknown mechanism(s) are induced by suckling. Prolactin release during suckling increased with increasing litter size and oxytocin administration attenuated the increase.

Introduction

During lactation, the suckling stimulus causes the release of prolactin from the anterior pituitary and oxytocin from the posterior pituitary. Oxytocin evokes milk ejection and prolactin stimulates the synthesis of milk components, such as casein and lactose, by the mammary gland. The actions of oxytocin and prolactin maintain lactation and the quantity of milk produced is determined by the suckling intensity of the number of pups attached to the nipples of mothers (Russell, 1980; Grigor *et al.*, 1984).

The concentration of lactose in plasma increases when the interval between milking is extended (Wheelock and Rook, 1966), at parturition and after weaning (Kuhn and Linzell, 1970;

Hartmann *et al.*, 1984; Matte *et al.*, 1989). The lactose content of the mammary gland decreases rapidly and the lactose concentration of the plasma increases coincidentally in response to oxytocin administration to lactating rats that had been isolated from their litter for 6 h (Yamamuro *et al.*, 1993). The administration of oxytocin also decreases the amount of lactose in milk in lactating cows (Wheelock *et al.*, 1965; Lane *et al.*, 1970; Graf *et al.*, 1973). It has been suggested that lactose leaks from milk in the mammary gland into the plasma, and that the mechanism by which this occurs depends on the opening of tight junctions between mammary epithelial cells in response to the accumulation of milk in the mammary gland during long intervals between milking, around parturition and after weaning (Pitelka *et al.*, 1973; Linzell and Peaker, 1974; Morgan and Wooding, 1982). A recent study has shown that when tight junctions in mammary glands are disrupted during extended milking intervals in goats, the plasma lactose concentration increases (Stelwagen *et al.*, 1994).

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The objective of the present study was to examine whether oxytocin administration induces the disruption of tight junctions in mammary glands as an index of the leakage of lactose from milk to plasma during suckling. The effect of oxytocin in rats suckling different numbers of pups on the suckling-induced release of prolactin and the milk ejection reflex in lactating rats were also examined.

Materials and Methods

Animals and treatments

Primiparous lactating rats of the Wistar strain were housed in individual cages at $22 \pm 1^\circ\text{C}$ under a photoperiod of 14 h light:10 h dark (lights on at 06:00 h). They were allowed free access to food and water. The litter was adjusted to eight pups on day 1 of lactation (the day after parturition). A permanent intra-atrial catheter was inserted into the mother on day 5 of lactation. Synthetic lyophilized oxytocin (Pitocin: Sigma Chemical Co., St Louis, MO) dissolved in saline was used in this study.

Experiment 1. At 09:00 h on day 12 of lactation, mothers ($n = 45$) were isolated from their litters. After isolation for 6 h, mothers were allowed to resuckle for 1 h; the size of the litter was adjusted to either two, eight or 12 pups. Oxytocin (1 iu in 0.1 ml saline) was inserted into the mothers through the catheter at the beginning of the resuckling period. Saline (0.1 ml) was infused via the catheter into the control rats. The difference in body mass of the litter before and after the 1 h suckling was used as an index of the amount of milk ejected from the mothers. Blood samples (300 μl) were taken through the catheter just before oxytocin injection (0 min), and 15, 30 and 45 min after injection of oxytocin to estimate the concentration of suckling-induced biologically active prolactin; an equal volume of saline was injected into the mothers after each blood sampling. Blood was collected by cardiac puncture under light ether anaesthesia at the end of the suckling period to measure the concentration of lactose in plasma.

Experiment 2. At 09:00 h on day 12 of lactation, mothers ($n = 24$) were isolated from their litters (eight pups), and the three most left-hand nipples of abdominal inguinal mammary glands of the mother were occluded by surgical adhesive agent. After being isolated from their pups for 6 h, mothers were allowed to resuckle with eight pups for 1 h. At the start of resuckling, 0.01, 0.1, 1 iu oxytocin (in 0.1 ml saline) or 0.1 ml saline was administered through the catheter. After the 1 h suckling period, blood was taken by cardiac puncture under light ether anaesthesia and the mothers were killed by decapitation.

Lactose in plasma and mammary glands

Blood taken from the mother by cardiac puncture was drawn into the heparinized tube, centrifuged at 1000 g for 20 min at 4°C , and the plasma obtained was stored at -30°C until

assayed for lactose. At the autopsy in Expt 2, occluded and unoccluded abdominal inguinal mammary glands were removed and weighed separately. The lactose in plasma and in mammary glands was determined by an enzymatic method (Harigaya *et al.*, 1978).

Concentration of biologically active prolactin in plasma

Blood (300 μl) drawn into a tube containing 300 μl heparinized (100 U heparin ml^{-1}) 0.01 mol PBS l^{-1} (pH 7.4) was centrifuged at 1000 g for 20 min at 4°C . The plasma was stored at -30°C and the biologically active prolactin concentration was assayed using the Nb2 rat lymphoma cell bioassay (Lawson *et al.*, 1982). In the assay, NIADDK rPRL-B-4 was used as the standard and the amount of plasma sample diluted with PBS was usually 50 μl . Each sample was assayed in duplicate and the mean was used as the representative value of the sample.

Statistical analyses

Statistical significance was based on the two-way analysis of variance and Duncan's new multiple range test. Student's *t* test was used to test for statistical significance between groups with the same number of pups. Values that were different at a level of $P < 0.05$ were considered significant.

Results

Experiment 1

The gain in body mass of individual pups in saline-injected rats suckling two pups during the 1 h suckling period was markedly lower than that of eight pups ($P < 0.01$) and 12 pups ($P < 0.001$; Fig. 1). A comparison between the saline-injected control and the oxytocin-injected groups suckling the same number of pups indicated that the litters of oxytocin-injected rats suckling two or eight pups were heavier than the equivalent control groups. In addition, the gain in body mass per pup in the group suckling two pups was significantly greater than those in the other two groups ($P < 0.01$ for eight pups and $P < 0.001$ for 12 pups). Also, the pups from the rats suckling eight pups had a greater mass gain than did the pups from the group suckling 12 pups. Thus, an inverse relationship was seen between the gain in body mass of a pup during the suckling period and the number of pups per litter in oxytocin-injected groups ($r = -0.859$; $P < 0.01$).

The increase in the litter size suckled resulted in a significant decrease in the concentration of plasma lactose in both oxytocin- and saline-injected groups (Fig. 2). When the plasma lactose concentration of oxytocin-injected groups was compared between groups of rats that suckled the same number of pups, the concentration was significantly higher ($P < 0.001$ for two pups and $P < 0.05$ for eight pups) than that of the saline-injected controls. Particularly, the concentration of lactose in rats suckling two pups increased by a factor of approximately 10 in response to oxytocin administration. The

Fig. 1. Effects of exogenous oxytocin (1 iu i.v.) to lactating rats suckled with different litter sizes on the gain in the mass of (a) litter and (b) individual pups (mean $\pm$ SEM) at the end of a 1 h suckling period after 6 h separation from their mother. Different capital letters (saline, □) and small letters (oxytocin, ■) indicate a significant difference between the values ($P < 0.05$). *, ***Values are significantly different between saline- and oxytocin-injected rats suckling the same number of pups ($P < 0.05$ and 0.001 , respectively). Numbers of rats tested are in parentheses.

Fig. 2. Effects of oxytocin (1 iu i.v.) administered to lactating rats suckled with different litter sizes on the concentration of lactose in the plasma of the mothers (mean $\pm$ SEM) after a 1 h suckling period following 6 h isolation from their litter. Different capital letters (saline, □) and small letters (oxytocin, ■) indicate a significant difference between the values ($P < 0.05$). *, ***Values are significantly different between saline- and oxytocin-injected rats suckling the same number of pups ($P < 0.05$ and 0.001 , respectively). Numbers of rats tested are in parentheses.

plasma lactose concentration in the group suckling 12 pups also tended to be higher in oxytocin-injected rats, but this increase was not statistically significant.

In the rats suckling two pups, the concentration of suckling-induced prolactin in plasma was not higher in either oxytocin-injected rats or saline-injected control rats (Fig. 3a). In the rats suckling eight pups, suckling gradually increased the concentration of plasma prolactin in both groups over time, but the increase was not significantly different in the oxytocin-injected group (Fig. 3b). In the rats suckling 12 pups the concentration of prolactin in plasma continued to increase following the initiation of suckling in both saline-injected and oxytocin-injected animals (Fig. 3c). However, in oxytocin-injected rats suckling eight or 12 pups, the increase in concentration of prolactin in plasma was lower than that in the respective

saline-injected rats. The prolactin concentration in rats suckled by 12 pups tended to be higher than in those suckling eight pups at all times measured.

Experiment 2

The mass of the mammary gland of the occluded side was not altered by any dosage of oxytocin (Fig. 4a). The difference between the mass of occluded and suckled mammary glands, which is considered to be equal to the amount of milk removed from the mammary glands by the litter, was greater in oxytocin-injected rats than in the control rats (data not shown). The amount of lactose in the occluded mammary glands was not significantly different among groups except for the group injected with 1 iu oxytocin (Fig. 4b). Administration of 1 iu oxytocin markedly decreased the amount of lactose in the occluded side and was significantly different ($P < 0.001$) from the other groups. In contrast, the amount of lactose in the suckled sides decreased in response to the administration of oxytocin in a dose-dependent manner. Concentration of lactose in the plasma of the rats injected with 1 iu oxytocin was significantly higher ($P < 0.05$) than that in the control rats and in those rats injected with 0.01 and 0.1 iu oxytocin (Fig. 5).

Discussion

The study has shown that suckling diminishes the leakage of lactose from milk to plasma by oxytocin administration in the lactating rat.

Reducing the litter from eight to two pups resulted in a loss of the body mass of the litter during a 1 h suckling period. This result indicates that a considerable reduction in the litter size does not induce the release of endogenous oxytocin from the posterior pituitary. The amount of oxytocin released appears to depend on the number of pups attached to the nipples (Fuchs and Wagner, 1963; Mena and Grosvenor, 1968), and the

Fig. 3. The concentration of suckling-induced biologically active prolactin in the plasma of rats (mean $\pm$ SEM) during the suckling period. Mothers were injected with oxytocin (1 iu i.v.) or saline and suckled by (a) two, (b) eight or (c) 12 pups. Different capital letters (saline, ○) and small letters (oxytocin, ●) indicate a significant difference between values ($P < 0.05$). *, ***Values are significantly different between saline- and oxytocin-injected rats suckling the same number of pups ($P < 0.05$ and 0.001 , respectively).

Fig. 4. (a) The mass and (b) the lactose content (mean $\pm$ SEM) of occluded (□) and suckled (■) mammary glands in lactating rats that had been injected with various doses of oxytocin and that had suckled eight pups for 1 h after 6 h of isolation from their litter. Different capital letters (occluded) and small letters (suckled) indicate a significant difference between values ($P < 0.05$). Numbers of rats tested are in parentheses.

threshold for the suckling-induced reflex release of oxytocin is, at least in part, set by the previous suckling experience of mothers (Wakerley *et al.*, 1978). A comparison between saline- and oxytocin-injected groups suckling the same number of pups indicated that the gain in litter mass in oxytocin-injected groups was significantly greater, or tended to be greater, and is due to the removal of alveolar milk. Moreover, reducing the number of pups suckled increased the gain in bodymass of an individual pup when oxytocin was administered. This result suggests that an individual pup in the groups suckling two or eight pups suckled from more than one nipple. The litter in the group suckling two pups could obtain milk only with the aid of exogenous oxytocin.

The amount of prolactin released from the anterior pituitary during suckling depends on the intensity of the suckling stimulus (Grosvenor and Mena, 1971; Grigor *et al.*, 1984). In the experiment reported here, the maternal biologically active plasma prolactin concentration also increased with increasing litter size, while the increase of prolactin in oxytocin-injected

groups was lower than that of the controls. One possible reason for the diminution of suckling-induced prolactin release by oxytocin administration is a decrease in the suckling intensity; this seems likely since litters could obtain milk more easily in response to exogenous oxytocin administration. There are some reports that a large dose of oxytocin decreases the release of prolactin from the anterior pituitary (Kuhn and McCann, 1970; Kuhn *et al.*, 1973). In addition, in the study reported here, the biologically active prolactin concentration in the plasma of oxytocin-injected rats suckling eight pups tended to be lower than that of the control, and in the oxytocin-injected rats suckling 12 pups the plasma prolactin concentration was significantly lower at all periods measured compared with those of the control groups. The results appear to confirm that the suckling-induced prolactin release was suppressed by exogenous oxytocin during the 1 h suckling period.

It has also been observed that prolactin release during a suckling period is delayed if oxytocin is injected, while fewer

Fig. 5. The concentration of lactose in plasma (mean $\pm$ SEM) in lactating rats that were injected with 0.01, 0.1 or 1 iu oxytocin and that suckled eight pups for 1 h after 6 h isolation from their litter. Points within a group with different letters are significantly different from each other ($P < 0.05$). Numbers of rats tested are in parentheses.

stretching reactions of litters did not occur in oxytocin-injected groups compared with those in control rats, and prolactin is normally released by the suckling stimulus after the efficacy of oxytocin administered has disappeared (Y. Yamamuro and N. Sensui, unpublished). Thus, it is possible that the decrease in suckling-induced prolactin release is a direct action of oxytocin. Recently, several researchers have reported that prolactin release is induced by oxytocin administration either *in vivo* or *in vitro*, and that the plasma concentration of suckling-induced prolactin is decreased by oxytocin antiserum (Lumpkin *et al.*, 1983; Samson *et al.*, 1986). Other studies, however, have shown that an oxytocin receptor antagonist (Johnston and Negro-Vilar, 1988) and oxytocin antiserum (Nagai *et al.*, 1983) abolishes the milk ejection reflex but does not affect concentrations of plasma prolactin. Thus, further investigation is needed to resolve whether oxytocin is a physiological regulator of prolactin.

The concentration of lactose in plasma remained low 6 h after removal of the litter (0.072 ± 0.032 mg ml⁻¹) and increased after 7 h (0.325 ± 0.150 mg ml⁻¹). This weaning duration is the critical period in the strain of rats used in this study when lactose in the milk begins to leak into the blood. The concentration of lactose in plasma in the saline-injected mothers that were suckled during the last hour of weaning tended to be lower compared with the value after 7 h without 1 h suckling. The results of Expt 1 indicate the following. First, in rats suckling two pups, milk in the mammary gland was not removed because of the lack of a sufficient suckling stimulus, as indicated by litter mass gain. Second, in rats suckling eight or 12 pups, milk was removed from the mammary gland by the pups; therefore, the amount of lactose in the plasma was low. In oxytocin-injected groups, the plasma lactose concentration of rats suckling two pups was markedly higher than in those suckling eight or 12 pups. This result indicated that the lactose in the nonsuckled mammary gland was transferred into the plasma. The removal of milk from most of the mammary gland by the numerous pups resulted in a lower concentration of lactose in the plasma.

In Expt 2, the amount of lactose in the mammary gland in the occluded side decreases in response to the administration of oxytocin at a dose of 1 iu to 21% of the amount of lactose in control rats, while 0.01 iu and 0.1 iu oxytocin did not affect the

amount of lactose. However, in a previous study (Yamamuro *et al.*, 1993) when mothers were injected with oxytocin at graded doses (0.01, 0.1 and 1 iu) without suckling after 6 h of isolation from their litter, a dose-dependent decrease in the lactose content of the mammary gland was observed (71%, 58% and 14% of that of the control, respectively). Allen (1990) also reported the same dose-dependent response to oxytocin in lactating cows. The difference between these reports and the present results indicates that suckling diminishes the transfer of lactose from mammary glands to the plasma. This suggests that unknown mechanism(s) other than the decrease in intra-mammary pressure by milk removal are involved, since the amount of lactose in occluded glands in rats administered 0.01 and 0.1 iu oxytocin did not decline even though milk was not removed. Oxytocin administration allows the pups to remove milk with a minimum amount of suckling stimulus, as evidenced by a lower concentration of prolactin in plasma; that is, oxytocin also diminishes the intensity of suckling by pups. In the lactating rabbit (Linzell *et al.*, 1975), treatment with prolactin increases the concentration of lactose in milk and $[K^+]$ in late lactation. It is possible that the permeability of mammary epithelium declines with a high concentration of prolactin in plasma. However, Hayden and Smith (1981) reported that the sensitivity of prolactin in the lactating rat to lactose synthetase activity in occluded mammary glands is lower than that in normal glands. Further investigation is needed to examine the possibility that exogenous prolactin administration alters the transfer of lactose from the mammary glands to the blood.

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