Integrity of the secretory epithelium of the lactating mouse mammary gland during extended periods after suckling

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Summary. The transepithelial potential difference (TEPD) and resistance (TER) in the alveoli, and the pressure in the mammary gland were measured in lactating mice at different times after they had finished suckling their young. Oxytocin administration caused a rise in TEPD, the amplitude of which decreased with time after cessation of the pups feeding. At 2·5 h after cessation, oxytocin administration caused a short-term decrease in TER, but at 5 h there was a two phase reaction: a fall in TER followed by a rise. At 20 h there was a one phase reaction, a rise in TER. TEPD in the mammary gland alveoli increased from its initial value of 18 ± 1 mV to 25 ± 1 mV at 2·5 h, and thereafter fell to zero by 15 h after suckling. The initial value of TER, 114 ± 1 kΩ, increased to 131 ± 4 kΩ at 2·5 h and then decreased by 5 h after suckling, but unlike TEPD it did not reach zero. These experiments show that extending the period after cessation of suckling does not cause a rise in intramammary pressure and that oxytocin injections cause a short-term rise in milk pressure. From these results, we propose a hypothesis of reducing ionic concentration gradients between intracellular fluid, cytoplasm of the secretory cell and milk at extended periods after cessation of suckling, starting at 2·5 h.

Experiments carried out mainly with such animals as goats and cows have shown that the integrity of the secretory epithelium of the alveoli of the mammary gland may be impaired as the period increases after the young finish sucking or milking ends. This is caused by increased pressure in the cavity of the alveoli, the excretory ducts, the cistern and the teats, and leads to lowering of the transepithelial potential difference (TEPD) (Neville & Peaker, 1981; Stelwagen *et al.* 1994). Animals such as goats and cows have relatively large vertically oriented cisterns, and it is possible to imagine the formation of vertical fluid (milk) columns, the pressure of which is apparently recorded by catheterization of the teat canal (Alekseev *et al.* 1992). In contrast, animals such as mice have no cisternal sections, so there is no anatomical basis for the formation of significant fluid columns. Hence a comparative physiological method is necessary for the study of the integrity of the alveolar section of the mammary gland and of the cellular regulation of secretory processes in lactating animals.

It is known that animals suckle their pups fairly regularly (Higuchi *et al.* 1986; Markov, 1992), so in the intervals between suckling secretion should accumulate in the gland. It has been shown that mouse mammary gland alveoli are characterized by characteristic values of TEPD and transepithelial resistance (TER), which change under different physiological influences (Tolkunov, 1989).

The aim of the present study was to investigate the integrity of the mouse mammary gland alveoli by measuring TEPD and TER in the alveoli together with the pressure in the mammary gland at different times after suckling had ceased.

materials and methods

All experiments were conducted with 60 white mice (Nursery 'Rappolovo', Leningradskaja region, Rappolovo 188667) weighing 30–40 g and suckling 8–10 young at about day 12–15 of lactation. The animals had free access to water and food. Before the experiments the animals were anaesthetized by intraperitoneal thiopental sodium injection (0·1 mg/g body weight).

It is known that for the mouse the maximum natural period between sucklings is 2·5 h (Markov, 1992). Dams were used immediately after suckling or after separation from their pups after the cessation of suckling for 2·5, 5, 10, 15 and 20 h. The minimum number of the animals in each time period was three. Pressure recording was performed on thoracic, abdominal and inguinal mammary glands. It was more convenient to record TEPD and TER on abdominal glands.

The experiments were conducted with the animals supine. For electrophysiological investigations the parenchyma of the mammary gland was separated from the adjacent skin layer. All the following manipulations were carried out with the aid of a stereoscopic microscope. TEPD was recorded with the help of sharpened glass microelectrodes (diam. 2–4 μm) filled with physiological saline (Tolkunov, 1989). The microelectrodes were pushed through the epithelial layer into the luminal cavity using micromanipulators. TER was measured with a pair of microelectrodes also filled with physiological saline inserted inside the alveolar cavity. The location of the tips of the microelectrodes in the alveolar cavity was controlled visually by squeezing the minimum quantity of physiological solution that could be seen.

To record the pressure in the mammary gland a pressure transducer (MPU 0·5; Nikon Co., Tokyo 100, Japan) was used together with an AP 611 amplifier. Preliminary investigations showed that minimum size for the polyethene catheter, filled with vaseline oil, necessary for recording pressure without distortion was i.d., 1·0 mm; o.d., 1·5 mm. The catheter used in the experiments was inserted inside the teat canal with the tip already cut. The inner cavities of the pressure transmitter and the catheter were filled with vaseline oil to provide fluid contact with milk.

All the experiments were carried out with the blood supply of the mammary gland preserved intact. For perfusion of the surface of the parenchyma of the mammary gland, the physiological solution was 153·8 mM-NaCl–5·6 mM-KCl–1·78 mM-$NaHCO_3$–2·16 mM-$CaCl_2$, pH 7·2–7·4 maintained at 37 °C (pH was adjusted by HCl or NaOH).

Synthetic crystalline oxytocin (OT, activity 450 i.u./mg; Institute of Organic Chemistry, Riga 226006, Latvia) was dissolved in the above physiological solution. When recording pressure the OT solution (100 μl, 5×10^{-4} i.u./ml) was introduced into the prepared femoral vein with the aid of a microscope (Tolkunov, 1989; Markov, 1992). In the electrophysiological experiments, OT was infused on to the surface of the mammary gland together with the perfused solution.

Results for the electrophysiological experiments are either shown for individual animals or reported as means $\pm$ SEM. Statistical differences between group means for control and experimental groups were assessed using Student's t test, taking $P < 0{\cdot}05$ as significant.

RESULTS

Effect of oxytocin on transepithelial potential difference and transepithelial resistance at different times after cessation of suckling

When recording TEPD and TER, absolute values for which are discussed below, we found that application of OT resulted in a rise in TEPD. It was possible to distinguish three types of reaction of TER: (1) a decrease, (2) an increase and (3) a two phase reaction consisting of an initial short-term decrease in TER (for 5–7 s) followed by an increase (Fig. 1). The proportions of these reactions varied with the period after cessation of suckling (Table 1). In general, with increasing time there were gradually fewer type 1 and more type 2 reactions. Two phase reactions were found in about the same proportions throughout.

These effects were accompanied by increases in TEPD at different periods after cessation of suckling for all animals ($P < 0{\cdot}01$ at all time points), although these varied considerably in magnitude (Fig. 2). Maximum changes in TEPD with OT infusion were found after 2·5 h, and with longer periods after cessation of suckling the increase in TEPD steadily decreased. Similar though less definite changes were observed in the duration of reactions caused by OT (Fig. 2).

Transepithelial potential difference and transepithelial resistance values in the mammary gland alveoli for extended periods after cessation of suckling

TEPD values in the mammary gland alveoli recorded in dams separated from their pups immediately before the experiment varied from -10 to -25 mV, the average value being -18 ± 1 mV. Milk was always negative relative to the surface of the gland (Fig. 3). After 2·5 h there was a consistent rise in TEPD ($P < 0{\cdot}01$), but at longer periods after cessation of suckling the value fell. At 15 h the value of TEPD was less ($P < 0{\cdot}01$). TEPD was close to zero in some alveoli: in 14% at 15 h, in 18% at 20 h. At these times there was a steep rise in the proportion of the alveoli with low (0–5 mV) TEPD: 83% at 15 h, 75% at 20 h.

The TER in animals separated from their pups just before measurement (controls) was 114 ± 1 kΩ (Fig. 3). After 2·5 h TER was 131 ± 4 kΩ: higher, but not significantly so. For longer periods, TER fell ($P < 0{\cdot}01$), and there were consistent differences from control values for animals separated from their pups for 10 h. Thereafter there was no further decrease in TER, and even for the longest periods tested TER could always be recorded in the alveoli (not falling below 40 kΩ). Of great interest was the TER value in those alveoli in which no TEPD could be measured. In spite of the absence of any TEPD in the alveoli, the TER varied between 40 and 70 kΩ. Hence, whereas there was a consistent correlation between TER and TEPD in animals tested immediately and at 2·5, 5 and 10 h after cessation of suckling ($r = 0{\cdot}5$, $P < 0{\cdot}01$, $n = 124$), there was no such correlation at 15 and 20 h ($r = 0{\cdot}1$, $P > 0{\cdot}05$, $n = 42$). These results showed that extending the period after cessation of suckling did not result in mechanical damage to secretory epithelium as a result of a possible milk pressure rise in the alveoli.

Recording of pressure in the mouse mammary gland

Pressure measurements showed that dams separated from their pups and tested immediately had intramammary pressures $< 0{\cdot}15$ kPa (1 mmHg), within the limits of experimental error and probably caused by the pressure of the tissue of the mammary gland itself. Extending the period after cessation of suckling did not produce any pressure rise in the mammary gland, even after 20 h. However, OT

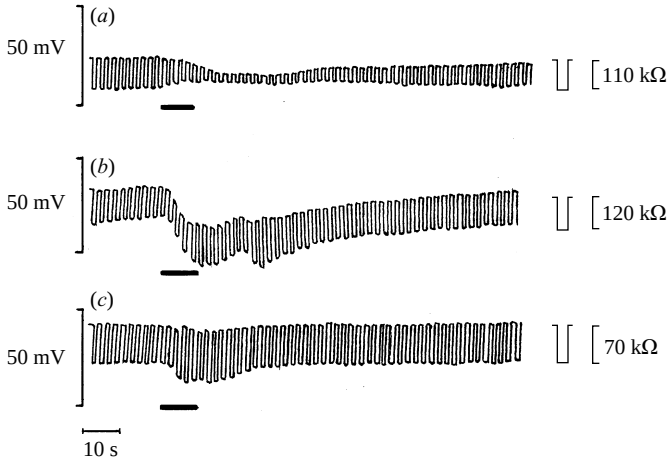

Fig. 1. Changes in transepithelial potential difference and resistance in lactating mouse mammary gland alveoli in response to the application of oxytocin solution (5×10^{-4} i.u./ml) at (*a*) 2·5, (*b*) 5 and (*c*) 20 h after they had ceased to suckle their young. ▬, Oxytocin application.

Table 1. *Types of response of transepithelial resistance in the mouse mammary gland alveoli to oxytocin application*

(Values are percentages of each type)

	Time, h					
Response	0	2·5	5·0	10·0	15·0	20·0
Decrease	14	100	66	30	10	8
Two phase†	43	—	34	40	30	—
Increase	43	—	—	30	60	92

† An initial decrease, followed by an increase.

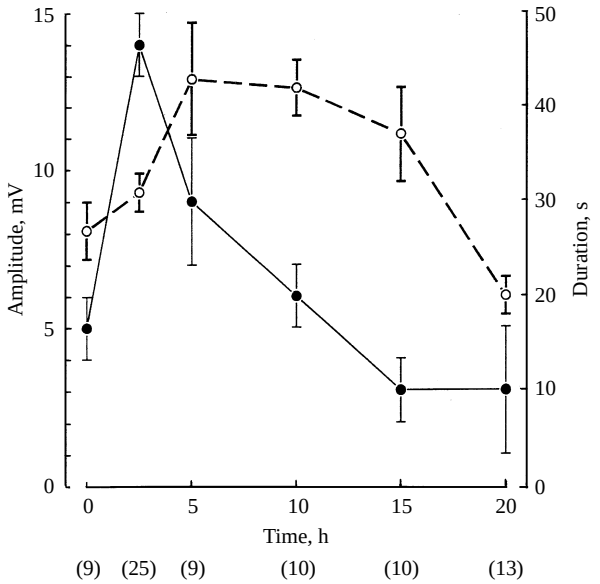

Fig. 2. Changes in ●, amplitude and ○, duration of the transepithelial potential difference in lactating mouse mammary gland alveoli in response to the application of oxytocin solution (5×10^{-4} i.u./ml) at different times after they had ceased to suckle their young. Values in parentheses are numbers of determinations.

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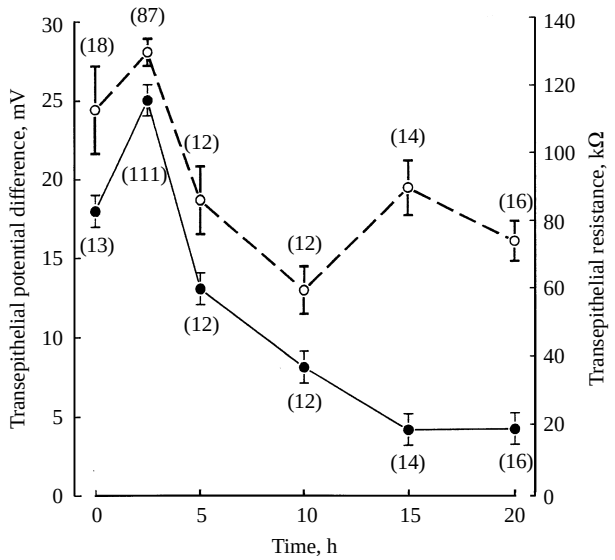

Fig. 3. Changes in ●, transepithelial potential difference and ○, transepithelial resistance in lactating mouse mammary gland alveoli in response to the application of oxytocin solution (5×10^{-4} i.u./ml) at different times after they had ceased to suckle their young. Values in parentheses are numbers of determinations.

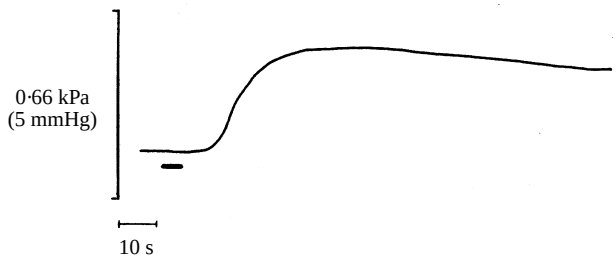

Fig. 4. Changes in intramammary pressure in the lactating mouse mammary gland after intravenous injection of 100 μl oxytocin solution (5×10^{-4} i.u./ml). ▬, Oxytocin injection.

injections produced pressure rises in the mammary gland of up to 0·40–0·66 kPa (3–5 mmHg); subsequently these returned to initial values (Fig. 4). Infusing large OT doses resulted in milk pressure rises up to 1·66 kPa (20 mmHg). Thus the pressure in the mouse mammary gland did not rise even after considerably extended periods following cessation of suckling, whereas OT injections resulted in the usual pressure reactions.

DISCUSSION

It is clear that the membrane potential of secretory cells and TEPD in the mammary gland reflect the activity of ionic processes. It may be noted that the membrane potential of secretory cells (32 ± 1 mV; Grachev *et al.* 1971) and TEPD in the mouse mammary gland alveoli (25 ± 1 mV; Tolkunov, 1989) are similar. Higher but also similar values (49 ± 2 and 35 ± 2 mV respectively) were recorded in another set of experiments on the mouse mammary gland (Berga, 1984). Electrophysiological and radioisotopic investigations lead to the conclusion that the apical plasma membrane of secretory cells of the mammary gland is permeable to K^+ and Na^+ ions in both directions (Linzell & Peaker, 1971; Berga & Neville, 1985). It has been shown that the potential difference across the apical plasma membrane is reversibly

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changed, in a predictable manner, when there are equal concentrations of Na^+, K^+ and Cl^- ions in milk and secretory cells (Blatchford & Peaker, 1988). According to an existing model of ion transport in the secretory epithelium of mammary gland alveoli (Peaker, 1977*b*; Maule Walker, 1984), the activity of the Na/K pump located in the basolateral plasma membrane plays a crucial role in generating concentration gradients for K^+ and Na^+ ions.

TEPD are present in the mammary gland secretory epithelium of such animals as goats (Blatchford & Peaker, 1988) and mice (Tolkunov, 1989) when cell junctions are tight (Berga, 1984). It should be also taken into account that ion and water fluxes can be via the paracellular pathway, controlled by the same tight junctions, which function as a selective barrier (Cereijido *et al.* 1989). It is accepted that the paracellular pathway of ion transport functions under conditions of increasing pressure or OT activity, causing TEPD to fall towards zero (Peaker, 1977*a*, 1980; Stelwagen *et al.* 1994). It is known that OT activity decreases TEPD in the goat mammary gland, but only if the initial intramammary pressure is relatively high, $> 4{\cdot}0$ kPa (30 mmHg). At lower pressures, OT increases the TEPD (Alekseev *et al.* 1992).

The formation of a shunt pathway for ion transport in response to OT is likely to be due to contraction of myoepithelial cells in the mammary gland (Moore *et al.* 1987; Müller *et al.* 1989). However OT also exerts a direct effect on the ionic permeability of plasma membranes of secretory cells of the mammary gland (Tolkunov, 1989). Analysis of ionic processes shows that hyperpolarization of the plasma membrane and increases in TEPD in the mammary gland alveoli are caused by Ca^{2+}-dependent K^+ channels (Tolkunov, 1989). Thus OT acts on both the myoepithelial and secretory cells of the mammary gland.

It is known that hyperpolarization of the plasma membranes of secretory cells and changes in TEPD are at least two component reactions, one of which reflects a rise in TER (Tolkunov, 1989). This is apparently caused by the physical influence of contracting myoepithelial cells in response to mechanical stimulation (Tolkunov, 1989). Moreover, mechanically activated ionic channels in the plasma membranes of secretory cells of the mammary gland are also possible (Furuya & Enomoto, 1990). Thus, the ionic permeability of plasma membranes of secretory cells and the secretory epithelium of the mammary gland alveoli can change under different external influences.

Analysis of the changes in TER indicates that the intensity of ion fluxes in the secretory epithelium decreases while its integrity is preserved. It has been shown that extending the period after cessation of suckling in mice produces a rise in Na^+ ions and a fall in K^+ ions in milk (Kisliakova & Leontiev, 1991). There are physiological adaptive mechanisms directed to reducing ionic concentration gradients and halting secretion. Therefore pressure rises in the mouse mammary gland can be prevented while at the same time ionic concentration gradients are reduced and the secretion of organic components such as lactose and proteins is inhibited.

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REFERENCES

Alekseev, N. P., Markov, A. G. & Tolkunov, Yu. A. 1992 Transepithelial potential difference in the goat mammary gland and its change during hand milking, and administration of oxytocin and catecholamines. *Journal of Dairy Research* **59** 469–478

Berga, S. E. 1984 Electrical potentials and cell-to-cell dye movement in mouse mammary gland during lactation. *American Journal of Physiology* **247** C20–C25

Berga, S. E. & Neville, M. C. 1985 Sodium and potassium distribution in the lactating mouse mammary gland in vivo. *Journal of Physiology* **361** 219–230

Blatchford, D. R. & Peaker, M. 1988 Effect of ionic composition of milk on transepithelial potential in the goat mammary gland. *Journal of Physiology* **402** 533–541

Cereijido, M., Ponce, A. & Gonzalez-Mariscal, L. 1989 Tight junctions and apical/basolateral polarity. *Journal of Membrane Biology* **110** 1–9

Furuya, K. & Enomoto, K. 1990 Real-time imaging of intracellular calcium change with simultaneous single channel recording in mammary epithelial cells. *Brain Research Bulletin* **25** 779–781

Grachev, I. I., Skopichev, V. G. & Tolkunov, Yu. A. 1971 [The effect of physiologically active substances on the membrane potential of the mammary gland's secretory cells.] *Fiziologicheskii Zhurnal SSSR imeni I. M. Sechenova* **57** 1046–1052

Higuchi, T., Tadokoro, Y., Honda, K. & Negoro, H. 1986 Detailed analysis of blood oxytocin levels during suckling and parturition in the rat. *Journal of Endocrinology* **110** 251–256

Kisliakova, L. P. & Leontiev, V. G. 1991 [The transepithelial transport of Na^+, K^+ and water in the lactating mammary gland of mice.] *Fiziologicheskii Zhurnal SSSR imeni I. M. Sechenova* **77** 130–136

Linzell, J. L. & Peaker, M. 1971 Mechanism of milk secretion. *Physiological Reviews* **51** 564–597

Markov, A. G. 1992 [Contractile responses of alveolar myoepithelial cells of the white mouse mammary gland to oxytocin.] *Fiziologicheskii Zhurnal imeni I. M. Sechenova* **78** 105–112

Maule Walker, F. M. 1984 Exocrine and endocrine secretions of the mammary gland: local control in ruminants. In *Physiological Strategies in Lactation*, pp. 171–191 (Eds M. Peaker, R. G. Vernon and C. H. Knight). London: Academic Press (*Symposia of the Zoological Society of London* no. 51)

Moore, D. M., Vogl, A. W., Baimbridge, K. & Emerman, J. T. 1987 Effect of calcium on oxytocin-induced contraction of mammary gland myoepithelium as visualized by NBD-phallicidin. *Journal of Cell Science* **88** 563–569

Müller, M., Soloff, M. S. & Fahrenholz, F. 1989 Photoaffinity labelling of the oxytocin receptor in plasma membranes from rat mammary gland. *FEBS Letters* **242** 333–336

Neville, M. C. & Peaker, M. 1981 Ionized calcium in milk and the integrity of the mammary epithelium in the goat. *Journal of Physiology* **313** 561–570

Peaker, M. 1977*a* Mechanism of milk secretion: milk composition in relation to potential difference across the mammary epithelium. *Journal of Physiology* **270** 489–505

Peaker, M. 1977*b* The aqueous phase of milk: ion and water transport. In *Comparative Aspects of Lactation*, pp. 113–134 (Ed. M. Peaker). London: Academic Press. (*Symposia of the Zoological Society of London* no. 41)

Peaker, M. 1980 The effect of raised intramammary pressure on mammary function in the goat in relation to the cessation of lactation. *Journal of Physiology* **301** 415–428

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

Tolkunov, Yu. A. 1989 [Ion-dependent hyperpolarization changes of membrane potential in secretory cells of the mammae.] *Fiziologicheskii Zhurnal SSSR imeni I. M. Sechenova* **75** 567–574