Effect of Rise in Plasma Osmotic Pressure on the Milk-ejection Reflex in the Rat

HIDEO NEGORO, KAZUMASA HONDA, TETSUJI FUKUOKA AND TAKASHI HIGUCHI

Department of Physiology, Fukui Medical School, Matsuoka, Fukui 910-11

Abstract

The effect of an increase in plasma osmolality on the milk-ejection reflex of rats was studied. The lactating rats, at day 8-12 of lactation, were anesthetized with urethane (1.1 g/kg, i.p.) and 8-11 pups which had been separated from their mother 16-18 h were put to the nipples to suckle. In 21 of 47 rats studied an intermittent pattern of milk ejection was recorded with a latency of 15-74 min (group-I rats). The mean interval between recurring milk ejections was 8.3±0.6 (S.E.M.) min and the mean amount of oxytocin released at each milk ejection estimated in 11 of them was 0.26±0.04 mU. The remaining 26 rats showed no milk ejection throughout the nursing period of more than 90 min (group-II rats).

The intraperitoneal injection of 1.5 M NaCl (1 ml) had no effect on the interval between milk ejections but increased the amount of oxytocin released at each milk ejection by 2.4 times in group-I rats, as the plasma osmolality changed from 297.9±2.7 mOsm/kg to 310.4±3.6 mOsm/kg. On the other hand, the 1.5 M NaCl injection induced recurring reflex milk-ejections in all of the group-II rats, while the plasma osmolality increased from 307±2.8 mOsm/kg to 320±3.1 mOsm/kg. The mean interval was not significantly different from that observed in the group-I rats. The sensitivity of the mammary gland to oxytocin was not altered by the 1.5 M NaCl injection. The injection of isotonic NaCl had no effect on the milk-ejection reflex.

These results indicate that an increase in plasma osmolality facilitates the milk ejection reflex in rats by increasing the amplitude but not by changing the rhythmicity.

The most established physiological role of oxytocin is to elicit milk ejection by stimulating the myoepithelial cells of the mammary gland to contract. In lactating animals milk ejection is naturally evoked by suckling of their young as a neuroendocrine reflex (Cross and Harris, 1952). The milk-ejection reflex of lactating rats exhibits many characteristics; milk ejection occurs intermittently at regular intervals during suckling, it is not induced with the onset of suckling, and even when milk ejection has started, there is no precise correlation between the intensity of suckling stimuli and oxytocin release (Lincoln et al., 1973). However, the central mechanism for the integration of suckling stimuli to form such characteristics of the milk-ejection reflex has not been clarified yet.

Andersson (1951) and Holland et al. (1959) have shown that the intracarotid injection of hypertonic solutions induces milk ejection without suckling stimuli and they postulated that not only vasopressin but also oxytocin was released by osmotic stimuli.
and that the oxytocin release was responsible for the milk ejection. Thereafter, Jones and Pickering (1969) observed that in the male rat water deprivation or salt loading depleted the pituitary oxytocin and Dogterom et al., (1977) found that water deprivation resulted in an increase in the plasma oxytocin concentration. More recently, an elevation of plasma osmolality by the intraperitoneal injection of hypertonic saline has been shown to increase plasma oxytocin in lactating rats (Brimble et al., 1978) and in male rats (Balment et al., 1980).

In the present study, we have examined the influence of increased plasma osmolality on the reflex milk ejection in lactating rats and attempted to investigate the nature of the interaction between suckling and osmotic stimuli.

A part of this work has been reported at a scientific meeting (Negoro et al., 1982).

### Materials and Methods

All experiments were performed in adult lactating Wistar rats (250-350 g, body weight). They were used for the experiment at day 8-12 of lactation. After 10-18 h separation from their young the mother rats were anesthetized with urethane (25% solution w/v) at 1.1 g/kg b.w., ip. The teat duct of an inguinal mammary gland was cannulated with a stainless steel tube (0.7 mm in outer diameter) and connected to a pressure transducer (Nihonkoden, LPU-0.1 A) for measurement of the intramammary pressure. The output from the transducer was plotted on a polygraph (Nihonkoden, RM-6000). A flexible cannula (Silastic, Dow Corning) was inserted into the right atrium through the right jugular vein to collect a blood sample for the determination of plasma osmotic pressure and to inject oxytocin (Atonin-O, Teikoku Hormone Mfg. Co.). Eight to 11 pups that had been separated from their mother for 16-18 h were applied to the nipples. Then, the rise in intramammary pressure was recorded as the index of milk ejection and the behavioral response of the pups was also monitored as the supplementary index. Mostly both indices were seen in parallel but in some cases the change in intramammary pressure was so small that the behavioral response of the pups was equivocal. About 90 minutes after the application of the pups, a blood sample (0.6 ml) was taken for the determination of plasma osmotic pressure from the cannula in the right atrium, and the same volume of isotonic saline was infused. Then, 1 ml of 1.5 M NaCl solution was injected ip as an osmotic stimulus and observation of the reflex milk-ejection was continued for another 90 min. A blood sample was again obtained 60-90 min after the hypertonic NaCl injection. The plasma osmotic pressure was determined by the method of freezing point depression using a Knauer Semimicro-osmometer. Several doses of oxytocin (0.1-1.0 mU) were injected through the atrial cannula before and after the hypertonic NaCl injection to examine the sensitivity of the mammary gland to exogenous oxytocin and to estimate, by matching, the amount of oxytocin released at each reflex milk ejection.

A pilot study was conducted in 4 lactating rats to examine the change in plasma osmolality induced by an ip injection of 1 ml 1.5 M NaCl. Blood samples (0.6 ml) were taken 0, 10, 20, 30, 40, 60 and 90 min after the 1.5 M NaCl injection. Each blood sample was immediately replaced by the same volume of isotonic saline. The injection of the hypertonic saline increased plasma osmolality within 10 min and the level of the osmolality significantly (p<0.01) higher than that of the control was maintained over a time course of 80 min (Fig. 1).

The statistical significance of the difference between two means was analyzed by paired t-test and the difference among three or more means was analyzed by analysis of variance and Duncan's multiple range test.

![Fig. 1. The change in plasma osmolality after ip injection of 1.5 M NaCl in urethane-anesthetized lactating rats. Each point with bar represents the mean±SEM of four determinations. *: Significantly different from control value (p<0.01).](image)
Results

Intramammary pressure during suckling

Four hours after anesthetization of the mother rats the pups were applied to the nipples of the mother and the intramammary pressure was recorded. Reflex milk ejections were elicited in 21 of the 47 lactating rats tested. There was a long latent period to the first milk ejection response. Subsequently a regular and intermittent milk ejection response occurred except when the mother rat showed a sign of arousal such as chewing, sniffing or attempts at righting; in such cases the milk-ejection interval was prolonged (Fig. 2). The latency to the first milk ejection was varied from 15 min to 74 min and the mean milk ejection interval was $8.3 \pm 0.6$ min. The amplitude of each milk ejection response was rather fluctuating. The amount of oxytocin released at each milk ejection was estimated in 11 rats to be $0.26 \pm 0.04$ mU.

The remaining 26 rats showed no reflex milk ejection over a recording period of 90 min, although suckling pups were continuously applied to the nipples. In 4 of them the observation period was extended to more than 180 min and yet no reflex milk ejection was recorded (Fig. 2). Thus the lactating rats tested were classified into two groups; the rats which exhibited a repetitive milk-ejection response to suckling stimuli within 90 min after application of pups (group-I) and those which displayed no reflex milk-ejection during the period of 90 min or more after application of pups (group-II).

Effect of isotonic NaCl on milk ejection

Three of the group-I rats and 5 of the group-II rats were tested for the effect of the ip injection of isotonic NaCl on the reflex milk-ejection. The group-I rats did not show any significant change either in the mean interval of the reflex milk-ejections or in the mean amounts of oxytocin released at each milk ejection. The group-II rats also showed no change; no reflex milk-ejection was elicited during the 90 min period after the injection of isotonic NaCl.

Effect of 1.5 M NaCl on milk ejection

The effect of an intraperitoneal injection of 1.5 M NaCl on the reflex milk ejection was investigated in 18 group-I rats and 21 group-II rats. Since the plasma osmolality was significantly higher than the control for at least 90 min after the ip injection of 1.5 M NaCl (Fig. 1), the intramammary pressure was observed during these 90 min. An example of the response of milk ejection to
1.5 M NaCl in a group-I rat and that in a group-II rat are shown in Fig. 3a and Fig. 3b, respectively. When 1.5 M NaCl was injected ip to group-I rats, the mean plasma osmolality was elevated significantly (p<0.01) from 297.9±2.7 mOsm/kg to 310.4±3.6 mOsm/kg, and the amplitude of the milk-ejection responses was increased but the mean interval between milk ejections was not changed significantly (Fig. 4a). On the other hand, all of the group-II rats tested started to display milk-ejection responses at mean intervals of 10.3±1.2 min as the plasma osmolality increased from 307.0±2.8 mOsm/kg to 320.4±3.1 mOsm/kg (Fig. 4b). The mean interval was not significantly different from that of milk ejections observed in group-I rats. The mean estimated amount of oxytocin increased significantly (p<0.05) from 0.26±0.06 mU to 0.74±0.15 mU (n=8) after 1.5 M NaCl treatment in the group-I rats, while that in group-II rats was 0.44±0.07 mU (n=12) (Fig. 5).

Eight group-I rats were tested for the effect of the removal of suckling pups from the mother. Either with or without 1.5 M NaCl treatment, the removal of pups led to an immediate cessation of milk ejection. When the pups were replaced after a 15 min interval milk ejections recurred with a mean latency of 20.75±7.21 min before the NaCl treatment and with a mean latency of 16.94±1.73 min after. The latency appeared to be shortened by the NaCl treatment but the change was not statistically significant.

![Fig. 3. Polygraphic records of intramammary pressure during suckling in the rats. a, Effect of ip injection of 1.5 M NaCl on milk ejections in the rat which displays milk ejections during control recording period. Note that the amplitudes of milk ejections markedly increase after 1.5 M NaCl, while the intervals between milk ejections do not change. b, Effect of ip injection of 1.5 M NaCl on milk ejections in the rat which displays no milk ejection during control recording period. Note that milk ejections are induced after 1.5 M NaCl.](image)

![Fig. 4. Effects of isotonic NaCl and hypertonic NaCl on plasma osmolality and milk-ejection interval. (a), The mean values in the rats which showed milk ejections during control period. (b), The mean values in the rats which showed no milk ejection during control period. The vertical lines indicate SEM. *: vs control p<0.01.](image)
Fig. 5. Effect of hypertonic NaCl on the mean estimate of oxytocin released at milk ejections in the rats which displayed milk ejections during control period (□; n=8) and in those which did not (■; n=12). The vertical lines indicate SEM. *: vs control p<0.05.

**Effect of 1.5 M NaCl on the sensitivity of the mammary gland to oxytocin**

Whether or not there was a change in the sensitivity of the mammary gland to oxytocin after 1.5 M NaCl treatment was examined in 4 group-I rats by comparing the responses of intramammary pressure to 0.5 mU of exogenous oxytocin. The mean intramammary pressures before and after 1.5 M NaCl treatment were 17.8±1.9 cm H₂O and 17.3±1.9 cm H₂O, respectively and there was no significant difference between them, indicating that the sensitivity of the mammary gland was not influenced by ip injection of 1.5 M NaCl.

**Discussion**

The present study showed that the elevation of plasma osmolality did not affect the frequency of reflex milk-ejections but increased their amplitude. Moreover, it induced reflex milk-ejections in rats which displayed no milk ejection in response to suckling stimuli in the lower osmolar state of plasma. Since osmotic stimuli had no effect on the sensitivity of the mammary gland to oxytocin, these findings indicate that an increase in plasma osmolality has a facilitatory effect on the milk-ejection reflex without affecting the rhythmicity of the reflex.

Electrophysiological studies on oxytocinergic neurones of anesthetized lactating rats have provided a detailed description of reactions of oxytocinergic neurones during suckling (Wakerley and Lincoln, 1973; Lincoln and Wakerley, 1974). No change in spike activity occurs after the attachment of pups until brief (2-4 sec) bursts of activity are observed at intervals of 5-10 min. Each burst is characterized by a 20-40 fold acceleration of firing. An abrupt rise in intramammary pressure follows the burst at a latency of about 15 sec. It has been suggested that there are two interrelated neural mechanisms in regard to the control of the milk-ejection reflex: a gating mechanism for fashioning the regular recurrence of the burst activity of oxytocinergic neurones and a mechanism of afferent summation for adjusting the size of the burst response (Lincoln and Wakerley, 1975). If these two mechanisms exist in the rat brain for the control of the milk-ejection reflex, the findings in the present study suggest that an increase in plasma osmolality may influence the latter mechanism but not the former.

However, Lincoln and Wakerley (1975) have also indicated in their unit study on paraventricular and supraoptic oxytocinergic neurones that the higher the background firing rates of a neurone the larger the burst response preceding the reflex milk-ejection is to be expected. The firing rates of oxytocinergic neurones as well as that of vasopressinergic neurones has been shown to increase as plasma osmolality increases (Brimble and Dyball, 1977), and the amount of oxytocin released following each burst response of the oxytocinergic neurones was found to be always proportional to the magnitude of the burst response (Lincoln and Wakerley, 1975).
Therefore, it is likely that an increase in the background activity of oxytocinergic neurones following an elevation of plasma osmolality may be responsible for the increase in the amplitude of the reflex milk-ejection observed in the present study. Furthermore, it can be speculated that even in the rats which displayed no milk ejection during suckling (group-II rat) a series of burst responses of oxytocinergic neurones might be evoked in response to the suckling stimuli, although the responses were too diminutive to display a detectable milk ejection and that, when background activity of the neurones was enhanced by an increase in plasma osmolality, the potential milk ejections might be disclosed. To examine this speculation an electrophysiological study on the interaction of suckling stimuli and osmotic stimuli at the level of oxytocinergic cells is now under way in our laboratory. Preliminary data indicate that what has been speculated is the case.

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References


