Impaired induction of prolactin secretion from the anterior pituitary by suckling in streptozotocin-induced diabetic rat

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Prolactin (PRL) secretion in streptozotocin-induced diabetic rats postpartum was examined to elucidate the reason for the reduced milk secretion of diabetic mothers. Pregnant Wistar rats were given citrate buffer (control group) or streptozotocin only (DM group) or with insulin (insulin group). Growth of pups was significantly lower in the DM group than in the control group, but similar in the insulin and control groups. Suckling-induced PRL secretion was significantly lower in the DM group than in the control group, and intermediate in the insulin group. TRH-induced PRL secretion was significantly lower in the DM group than in the control group, but the same in the insulin and control groups. Histologically, the mammary glands in the DM group were relatively less developed than those in the control group. The results suggest that reduced milk secretion in diabetic mothers is due to impaired induction by suckling of PRL secretion from the anterior pituitary as well as poor development of the mammary gland.

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Mammary growth, lactogenesis and maintenance of lactation are controlled by the coordinated secretions of a variety of hormones (1) and disturbance of this coordination results in decreased lactation.

Miyake et al. reported that the amount of suckled milk was significantly reduced in diabetic women (2), but the reason for this reduction in milk volume in diabetes is unknown.

In the early postpartum period, milk production correlates well with the postsuckling increase in PRL (3). If pituitary function is disturbed in diabetic mothers, the postsuckling increase in PRL may be impaired, and the milk volume reduced.

Insulin is known to affect the utilization of several key nutrients, including glucose. Moreover, it can promote epithelial cell proliferation and is required for conversion of non-secretory mammary cells to secretory cells in the presence of glucocorticoid and PRL (4–6). Thus insulin is essential for the maintenance of normal lactation: indeed, insufficient insulin secretion would reduce the synthetic capacity of the mammary gland (7).

On the other hand, a substantial decrease in TRH-induced PRL release was observed in streptozotocin-induced non-pregnant diabetic rats (8). Since TRH acts directly on the pituitary (9, 10), disturbance of pituitary function may be involved in the reduced lactation in diabetic mothers.

Physiological doses of insulin are reported to increase PRL production and the PRL mRNA level (11, 12). Thus insulin may have a role in suckling-induced PRL secretion from the anterior pituitary.

In this study, we found that pituitary secretion of PRL during lactation is impaired in streptozotocin-induced diabetic rats, but is partially restored by insulin administration.

Material and methods

Animals

Female Wistar rats. 60 days old, weighing 200–250 g, kept in a room at constant temperature (25°C) and humidity (50%) with lights on from 08.30 to 20.30 h were provided with Purina rat chow and water ad libitum. The female rats were paired with fertile males, and smears were checked for the presence of sperm the following morning (day 1 of pregnancy). The animals were followed-up throughout pregnancy, delivery and early postpartum. The litter size was adjusted to 10 pups on the first postpartum day.

Streptozotocin and insulin injection

The protocol of the experiment is shown in Fig. 1. Streptozotocin was purchased from Wako Pure Chemical Industries (Osaka, Japan). On day 7 of pregnancy, it (60 mg/kg bw) was injected, freshly prepared in 10 mmol/l citrate buffer containing 0.15 mol/l NaCl (pH 4.5), into the femoral vein (DM group). The control group received a single injection of citrate buffer on day 7 of pregnancy. Some of the diabetic rats received isophane insulin (4–12 unit/rat/day) as two daily subcuta-
neous injections (one at 09.00 h the other at 18.00 h) in the back from day 9 of pregnancy (48 h after streptozotocin injection, when the diabetic state was well established) to day 11 postpartum (insulin group). Each group had 20 rats. In the insulin group, blood glucose was measured twice a day so that the amount of insulin could be regulated to achieve almost normal blood glucose level. In the other two groups, blood glucose was determined at least twice a week. In animals of the DM group it was more than 22 mmol/l, in the control group about 6 mmol/l and in the insulin group about 6–12 mmol/l throughout the experiment. Blood glucose was determined from the tail vein using a Glucoster kit from Miles Laboratories (Elkhart, Indiana, IN) by a method based on the glucose-oxidase-peroxidase reaction.

**Weight of pups**

Lactational performance was assessed by weighing the pups in the three groups daily from birth to postnatal day 10.

**Suckling-induced PRL secretion**

On day 10 postpartum, rats were anaesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL) (50 mg/kg ip) and a jugular vein was cannulated, exteriorizing the cannula through the back of the neck. Unskillful surgery brought about the deaths of half of the animals from this procedure. On day 11 postpartum, the pups were separated from their mothers and from 3–4 h later blood samples (300 µl) were collected every 10 min and replaced by an equal volume of saline. After a 30-min control period the pups were reunited with their mothers for 40 min and then removed; blood collection was continued for an additional 50 min. The blood was centrifuged (1000 × g for 5 min) and the serum stored frozen until PRL assay.

**PRL response to TRH injection**

After examination of suckling-induced PRL secretion, the PRL response of lactating rats to TRH injection was examined. For this, the rats received an iv injection of 4 µg/kg bw of TRH (TRH Injection Tanabe, from Tanabe, Osaka, Japan). Blood samples (300 µl) were collected at 5-min intervals before and until 20 min after TRH injection.

**Radioimmunoassay (RIA) of PRL**

PRL was determined in duplicate with an RIA kit kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Rat Pituitary Hormone Distribution Program. Rat PRL RP-3 was used as a reference preparation. The inter- and intra-assay cvs were 7.2% and 5.1% respectively.
Statistical analyses

Statistical analyses were performed by two-way analysis of variance (ANOVA) for repeated measurements combined with Tukey’s post hoc test. Data on the maximum increment of PRL (APRL) after TRH injection were analysed using Student’s t-test unpaired.

Histological examination of the mammary glands

To clarify the effect of insulin deficiency on the mammary development, we examined the mammary glands of the three groups of rats histologically. The abdominal mammary gland on one side in all three groups was collected on day 1 postpartum and fixed in 10% formalin. After fixation, it was cut and embedded in paraffin. Sections of all the strips were stained with hematoxylin and eosin (HE). Each specimen was examined histologically in light microscopy.

Results

Effect of diabetic state on the growth of pups

Fig. 2 gives the mean weights of pups in the three groups from postnatal days 1 to 10. Some of the rats were excluded from the analysis of data because weighing of their pups took 10 days to complete and there were some missing data. Animals in the three groups produced similar numbers of pups, whose body weights at birth were similar. From postnatal day 5, the weight gain of pups was significantly lower in the DM group than in the control group, but not significantly different in the insulin and control groups. The mean body weights of pups in the control, insulin and DM groups were 18.1±0.5 g, 17.0±0.3 g and 12.3±0.5 g, respectively on postnatal day 10. During the first 10 days of the lactation period, the mean survival rate of pups was 91.2% in the DM group and 98.2% in both the control and insulin groups. To determine whether the cause of decreased weight gain and lower survival of pups in the DM group was due to a difference in the mothers or the pups, we carried out a cross-fostering experiment. We found that pups of the DM group rats nursed by the control group rats grew similarly to pups born and nursed by control group rats. And we also found that pups of the control group rats nursed by the DM group rats grew similarly to pups born and nursed by the DM group rats. These findings indicated that mother rats in the DM group were unable to supply adequate milk, which is the sole source of nutrients for the pups. On the contrary, rats in the insulin group had almost the same ability as control rats to secrete milk.

Suckling-induced PRL secretion

The PRL secretions induced by suckling in the three groups are shown in Fig. 3. In the control group, the serum PRL level increased from 85–95 μg/l to 1086.0±181.5 μg/l (mean ± SEM) during suckling for 30 min; on removal of the pups it promptly decreased to the presuckling level. In the DM group, the PRL level was 30–40 μg/l initially and increased to 123.2±27.7 μg/l during suckling for 30 min. This suckling-induced PRL secretion was significantly (p<0.01) less than that in either the control or the insulin group. The profile of PRL secretion in the insulin group was intermediate between those in the control and DM groups. In the insulin group, the PRL level reached a peak of 587.5±95.3 μg/l during suckling for 20 min, but the response was significantly (p<0.01) less than that in the control group.

Fig. 2. Body weight of pups in the control, insulin and DM groups. Values are means±SEM. ○ control group (N=9); □ insulin group (N=19); ● DM group (N=14). *p<0.01 (vs value of DM group by two-way ANOVA with Tukey’s post hoc test).

Fig. 3. Suckling-induced increase of PRL secretion in the control, insulin and DM groups. Values are means±SEM. ○ control group (N=10); □ insulin group (N=14); ● DM group (N=11). *p<0.01 (vs value of insulin group by two-way ANOVA with Tukey’s post hoc test).
PRL response to TRH injection

The responses of PRL to intravenous injection of TRH in the three groups are shown in Fig. 4. The maximum increment of serum PRL (ΔPRL) after TRH injection was significantly (p<0.01) lower in the DM group (43.9±16.4 μg/l) than in the control (111.9±10.9 μg/l) and insulin groups (149.4±42.1 μg/l). The ΔPRL did not differ significantly between the control and insulin groups.

Histological examination of the mammary glands

The mammary glands of the control group were characterized by a well-developed delineation of the interlobular connective tissue, so the lobuloalveolar structure was well defined (Fig. 5a). The lumen of the alveoli was wide and the walls dilated and thin. Many epithelial cells contained small droplets of fat (Fig. 5b). These findings reflected good secretory activity of the mammary glands. By contrast, delineation of the interlobular connective tissue in the DM group was hardly definable, so the lobuloalveolar structure seemed fairly immature (Fig. 6a). The alveoli were well distended, however, and small droplets of fat were present in many epithelial cells (Fig. 6b), showing almost the same secretory activity of the mammary glands as those in the control group. In the insulin group, histological characterization of the mammary glands was similar to that of the control group.

Discussion

In this experiment on pregnant rats we observed an impaired growth of pups born to and nursed by DM group rats. In contrast, pups of insulin-treated DM rats (insulin group) grew almost as well as pups of control rats. Body weight gain is thought to be a quantitative measure of lactational performance in rats (13). Thus this finding suggests that in the DM group milk supply to the pups was insufficient, as observed in diabetic women (2), and that insulin treatment restored it.

Streptozotocin is reported to cross the placenta and to be toxic to the fetus (14), which might therefore be diabetic, but pups of diabetic mothers grew normally when nursed by the control mothers. Though there was the possibility that insulin in the milk of the control mother will be absorbed by the diabetic pups and will cause normalization of growth, the impaired growth of pups nursed by diabetic rats was thought to be mainly
due to a deficiency in their mothers, most probably reduced milk secretion as a result of inadequate secretion of insulin, because pups of the control mothers which are not diabetic became retarded when nursed by the DM mothers.

Several causes for low milk yields in the diabetic state are conceivable. In this study, first we examined the PRL secretion on suckling.

PRL is reported to be the main factor determining the initiation and maintenance of lactation (15). It acts directly on the mammary alveoli to promote the synthesis and secretion of milk proteins (15). Aono et al. reported a close relationship between release of PRL and milk production in early puerperal mothers (3). Therefore, if the postsuckling increase is impaired, the milk volume may be reduced. In the present study, suckling-induced PRL secretion was significantly lower in the DM group than in the control group and intermediate in the insulin group. Thus, insulin deficiency reduced the response of PRL secretion, and supplement of insulin to diabetic rats resulted in partial recovery of this response. These data suggest that insulin may play an important role in the suckling-induced PRL secretion reflex, and impairment of this reflex in the insulin deficiency state may lead to low milk yield in the diabetic mother. The levels of PRL prior to suckling in the three groups were very high, one reasonable explanation being that the surgical stress may have been influenced. Furthermore, the base level of PRL in the insulin group was relatively high compared with the other groups. These might have been stressed by our sampling of tail blood and by their receiving an sc injection of insulin twice a day in addition to the surgical stress.

Suckling-induced PRL secretion is a classical neuroendocrine reflex. Stimulation of the nipples by suckling results in a marked increase in the serum concentration of PRL (16, 17). Suckling triggers nerve impulses from sensory receptors in the nipples, which ascend in the spinal cord and pass through the midbrain to the hypothalamus (18, 19). Mediated by serotonin, the response affects the tuberohypophyseal branch of the dopamine (DA) system. Suckling suppresses DA release and stimulates secretion of the PRL releasing factor (PRF) from the posterior pituitary, which in turn stimulates PRL secretion from the anterior pituitary (18, 20). To determine which site of this neuroendocrine reflex route is impaired, we examined the pituitary function of postpartal rats by TRH injection, because specific receptors for TRH are present in the lactotropes (19) and TRH stimulates PRL release from the anterior pituitary (9, 10). TRH controls the phase of release of PRL, whereas DA controls the depletion-transformation phase in the anterior pituitary (1). We observed a reduced PRL response to TRH in the DM group and complete recovery of the response in the insulin group. Thus insulin deficiency probably disturbs the pituitary function and impairs the phase of PRL release in the anterior pituitary.

These observations support the notion that insulin has a role in the phase of release of PRL in the anterior pituitary on some stimulations. It has also been demonstrated that insulin increases the relative levels of PRL mRNA sequences in both GH3 cells and normal rat pituitary cells (11, 12). Thus insulin may be involved in the phase of both synthesis and release of PRL.

Insulin treatment resulted in complete recovery of TRH-induced PRL secretion by diabetic rats, but only partial recovery of this suckling-induced PRL secretion. The reason for this discrepancy is not clear.

As mentioned above, an impaired pituitary function to secrete PRL may be one reason for low milk yield. However, milk production in the postpartum period is
also influenced by development of the mammary glands during pregnancy. So another reason for the low milk yield may be ascribed to the poor mammary development. Insulin is one of the important mammmogenic factors and is required for the development and proliferation of the mammary glands (4–6). Insulin was also reported to be essential for the accumulation of casein mRNA (21, 22). Maintenance of low circulating insulin levels resulted in an irreversible decrease in the number of mammary alveolar cells (23).

It has been reported that until around the delivery, the effect of prolactin on the differentiation of mammary glands was inhibited, since high estrogen and progesterone levels reduced the number of mammary prolactin receptors (24, 25). Therefore, it seemed appropriate to study the effect of insulin on the differentiation of mammary glands in this period.

In the histological study of rat mammary glands on day 1 postpartum, we found that the lobuloalveolar structure in the DM group was fairly immature, compared with that of the control and insulin groups. Though the secretory activity on day 1 postpartum did not differ histologically among these groups, the inadequate mammary development could have affected the decreased milk secretion in the DM group from day 5 postpartum. Therefore, poor development and proliferation of the mammary glands due to insufficient insulin secretion may be another reason for the reduced milk volume in streptozotocin-induced diabetic rats.

We conclude from these results that the reduced milk volume in streptozotocin-induced diabetic rats is probably due to impaired function of suckling-induced PRL secretion from the anterior pituitary, as well as poor development of the mammary glands.

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