Effect of Estrogen on Hyperprolactinemia-Induced Glucose Intolerance in SHN Mice (44012)

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Abstract. The effects of prolactin (PRL) on circulating levels of glucose and insulin, and of estradiol on hyperprolactinemia-induced glucose intolerance of tissues were studied in pituitary-grafted SHN mice (PG mice) and sham-operated controls. Pituitary grafting (PG) decreased blood glucose levels in male mice at 1 and 3 months after the operation but did not alter those in females. PG had little effect on serum insulin levels in males, but increased those in females. In female mice at 2 months after PG, blood glucose levels were significantly higher at 1, 2, and 4 hr after glucose load when compared with those in controls. In contrast, there was no significant difference in blood glucose levels after glucose load between male PG and control mice. The rate at which blood glucose levels decreased was slower in female PG mice than in controls during the 30 min after insulin injection, whereas there was no difference in the rate after insulin injection between male PG and control mice. In ovariectomized (Ovx) mice, no significant difference was found in the blood glucose levels after a glucose load between PG and control groups at 2 months after PG. In Ovx mice treated daily with estrogen, however, a PG-dependent high level of blood glucose was observed after glucose load. These results suggest that hyperprolactinemia decreases glucose tolerance *via* an increase in insulin resistance in female SHN mice and that estrogen is essential to the expression of the PRL effect. [P.S.E.B.M. 1996, Vol 212]

While prolactin (PRL) stimulates the proliferation and insulin secretion of pancreatic islet B cells (1–3), it induces insulin resistance in many extramammary tissues (4, 5), and hyperprolactinemia causes impaired glucose tolerance (6, 7). These effects of PRL are responsible for the coordinated control of metabolism to mobilize nutrients for milk production by the mammary glands. Thus, during lactation in rodents, an increase in the circulating level of PRL is considered to trigger "homeorhesis," meaning orchestrated changes for the priorities of a physiological state, namely lactation (8, 9). This PRL role is not achieved without the synergistic actions of other hormones. For example, the development of mammary gland and milk production require not only PRL but also insulin, growth hormone, estrogen, and cortisol (10, 11).

Pituitary grafting (PG) provides an useful experimental model with which to study the long-term effects of homologous PRL on metabolism (12, 13). We found that proliferation is enhanced in pancreatic islet B cells and that fat deposition in adipose tissues is decreased in pituitary-grafted mice (PG mice) (1, 8, 14, 15). However, the effect of PG on blood glucose levels differed with sex; PG resulted in hypoglycemia in males but not in females. These results implied that the PRL effect on glucose metabolism is modified by other hormones such as sex steroids. It is unknown whether the sex difference in the PRL effect upon glucose metabolism is caused by a difference in tissue sensitivity to insulin or by a difference in the ability of B cells to secrete insulin.

In this study, we analyzed changes in blood glucose levels after glucose or insulin injection in PG and sham-operated control mice to clarify sex differences in the PRL effect on glucose metabolism and sensitivity to insulin. In addition, ovariectomy was performed or estrogen was administrated to determine the contribution of estrogen to the effect of PRL.

Materials and Methods

Animals. Seven-week-old mice of the SHN strain (16) were housed in plastic cages with wood shavings under controlled temperature (25° ± 0.5°C) and light (12 hr of light from 6:00 to 18:00). They were given a commercial diet (CE-7; CLEA Japan Inc., Tokyo, Japan) and tap water *ad libitum*. All procedures used on the mice were approved by the Animal Care and Use Committee of the Graduate School of Science, University of Tokyo.

Hyperprolactinemia was induced by transplanting a single anterior pituitary gland obtained from litter mates of the opposite sex under the left kidney capsule as described (17). Sham-operated mice were also prepared as the controls. Ovaries of some mice were dissected out at the same time of pituitary grafting. Half of the ovariectomized mice were given subcutaneous injections of 0.25 μg of estradiol-17β in 0.1 ml of sesame oil twice daily for 2 months after the operation (Ovx + E_2 mice), and the other half received the vehicle only (Ovx mice).

Glucose and insulin determination. Blood glucose and serum insulin levels were determined as previously described (1, 8). One drop of blood was collected from the tail vein to determine the blood glucose level by a glucose oxidase method using a kit (Serapaper; Eiken, Tokyo). To determine the circulating insulin level, blood samples were collected from the trunk. Serum was separated and stored at −70°C. Insulin levels were determined by an enzyme immuno-assay (Glazyme Insulin EIA-Test kit; Wako, Tokyo, Japan).

Glucose or insulin load. For the glucose load study, each mouse was fasted for 16 hr, then given a single intraperitoneal injection of glucose (1 g/10 ml saline/kg body wt) or the vehicle between 10:00 and 12:00, and blood glucose levels were measured 0.5, 1, 2, and 4 hr later (18). To study glucose load in the Ovx and Ovx + E_2 groups, blood glucose levels were measured just before and at 2 hr after the glucose injection.

Insulin sensitivity was determined by a simple insulin tolerance test (19) with a slight modification. Mice fasted for 3 hr were given a single injection of ovine insulin (0.3 IU/10 ml saline/kg body wt; Sigma Chemical Co., St. Louis, MO) *via* the tail vein. The blood glucose levels were measured just before and 30 min after the injection. One day before the study, the mouse was injected with vehicle after a fasting for 3 hr and the blood glucose level was also measured before and 30 min after the injection. The net decrease rate in the blood glucose level was obtained by subtracting the decreased amount 30 min after insulin injection from the value after vehicle injection. Insulin sensitivity is expressed as net decrease rate per minute.

Statistical analysis. For statistical analysis, n in parentheses represents the number of samples. The statistical significance of differences between PG and control groups was assessed by Student's t test.

Results

Glucose and insulin levels in PG mice. Blood glucose and serum insulin levels were measured in male and female mice at 7 weeks of age, and at 1 and 3 months after PG (Fig. 1). In female mice, blood glucose levels were little altered by PG, while the serum insulin level was significantly increased at 3 months after PG. On the other hand, in male mice, the blood glucose level was markedly decreased after grafting, while serum insulin level was little affected.

Glucose or insulin load in PG mice. Figure 2 shows the blood glucose levels after glucose injection in PG and control mice at 2 months after PG. In sham-operated control mice of both sexes, glucose injection increased blood glucose levels, which was followed by a rapid decrease, within 2 hr, to the ordinary level. In female PG mice, a glucose injection increased the glucose levels in the same manner as it did in control mice during the first 30 min. However, the high glucose levels were maintained for 4 hr after the injection. Thus, PG decreased glucose tolerance in female mice. On the other hand, no delay of the recovery of blood glucose level was observed in male PG mice after
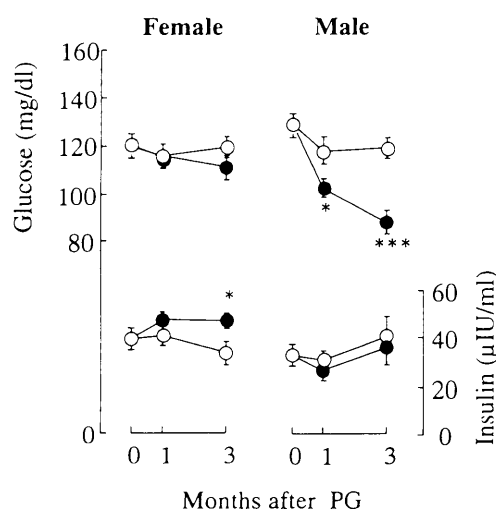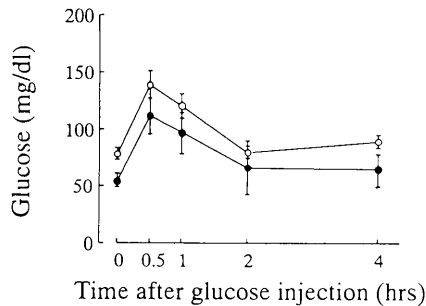

Figure 1. Blood glucose and serum insulin levels in PG (●) and control (○) mice. Vertical bars indicate means ± SEM (n = 10–16). *$P < 0.05$; ***$P < 0.001$.
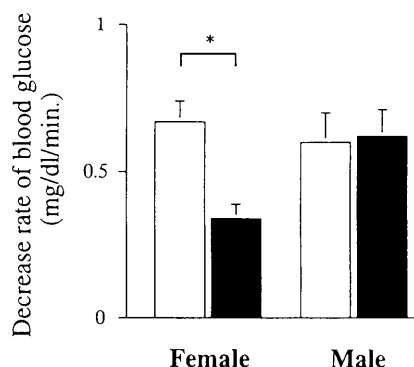
Female
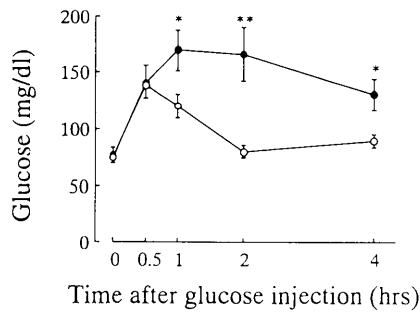
Male

Figure 2. Blood glucose levels after a glucose load in PG (●) and control (○) mice. Vertical bars indicate means ± SEM ($n = 8$). *$P < 0.05$; **$P < 0.01$.

glucose injection. A saline injection did not affect the blood glucose levels of any group (data not shown).

Net decrease rate in blood glucose level by insulin injection are shown in Figure 3. In females, the decrease rate was significantly smaller in PG mice than in controls, suggesting that PG desensitized insulin action at the tissue level in female mice. There was no difference in the decrease rate by insulin injection between male PG mice and controls.

Glucose load in ovariectomized PG mice with or without estrogen treatment. Figure 4 shows changes in blood glucose level 2 hr after glucose load in PG and control mice of Ovx or Ovx + E_2 group at

2 months after PG. The results are expressed as the values relative to blood glucose levels just before glucose injection. As described above, PG resulted in a retention of high blood glucose levels in intact females after a glucose load (Fig. 2). In Ovx mice, however, there were no significant differences in glucose levels after a glucose load between PG and control groups. On the other hand, among Ovx + E_2 mice PG induced high blood glucose levels after glucose load. Thus, estrogen is considered necessary for the PG-induced expression of a high blood glucose level after a glucose load.

Discussion

PRL stimulates insulin secretion from pancreatic islets (2, 3). In male mice, PG causes hypoglycemia associated with little change in serum insulin level, which implies an increase in the tissue sensitivity to insulin accompanying the possible stimulation of insulin secretion by PRL (8, 20). On the other hand, in female mice, PG resulted in hyperinsulinemia with no change in the blood glucose level. This implies that PRL increases resistance to insulin associated with enhanced insulin secretion. In fact, recovery from a high level of blood glucose after a glucose load was retarded in PG females, suggesting that PRL decreased glucose tolerance in female mice. As insulin is the most potent hormone involved in recovery from a high level of blood glucose (21), the impaired glucose tolerance in hyperprolactinemic female mice may be due to either a decrease in insulin secretion or a decrease in tissue sensitivity to insulin. The insulin load study revealed that hyperprolactinemia desensitized insulin action at tissue levels in female SHN mice. Hence, the impaired glucose tolerance in the hyperprolactinemic female SHN mice may be mainly due to a decrease in tissue sensitivity to insulin, although Hawkins *et al.* reported (22) that hyperprolactinemia induces diabetes
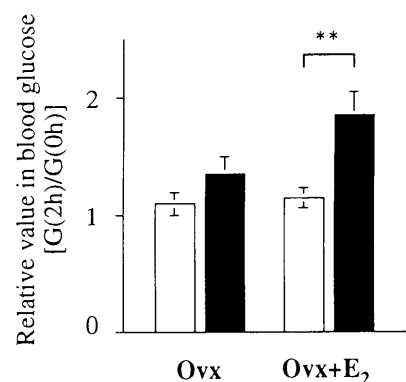

Figure 3. The rate at which blood glucose levels decrease during 30 min after an insulin injection in PG (black column) and control (clear column) mice. The values are expressed per minute as net decrease rates. Vertical bars indicate means ± SEM ($n = 10$). *$P < 0.05$.

Figure 4. Effect of PG on glucose tolerance in Ovx and Ovx + E2 mice. Relative values of blood glucose levels 2 hr after a glucose load (G[2h]) to those immediately before (G[0h]) were measured in PG (black column) and control (clear column) mice in the Ovx or Ovx + E_2 groups. Vertical bars indicate means ± SEM ($n = 6$–8). **$P < 0.01$.

due to autoimmune destruction of the pancreatic islets in the nonobese diabetic mouse.

The delayed recovery from a high level of blood glucose after glucose load was PG-dependent in intact and estrogen-treated ovariectomized females, but not in ovariectomized females nor in intact males. Although we did not measure the blood PRL levels here, PG was expected to induce hyperprolactinemia with the same manner in all groups of mice despite the difference in the blood levels of sex steroids (23). Hence, the present results suggest that estrogen is essential for the effect of PRL on tissue sensitivity to insulin.

Insulin stimulates lipogenesis and inhibits lipolysis in adipose tissues (21). PG has a lipolytic effect on epididymal adipose tissues despite small changes in the insulin levels in both sexes of mice (8), suggesting that PRL decreases the tissue sensitivity to insulin in adipose tissues irrespective of the circulating level of sex steroids. The PRL effect on the tissue sensitivity to insulin or the modulation of estrogen to the PRL effect may differ with tissues. It is unknown which tissue is responsible for the estrogen-dependent effect of PRL on insulin resistance. Tissue sensitivity to insulin in the liver and/or skeletal muscle may be involved, since these tissues are the major sites of insulin action in the disposal of an oral glucose load (21). In fact, skeletal muscle is insulin resistant during lactation (24), and hyperprolactinemia alters the activity of hepatic enzymes related to glucose metabolism in rats (25). Increased utilization of lipids, instead of glucose as the energy source, in the liver can be a cause of the insulin resistance in hyperprolactinemic mice (26).

Here, we used PG-induced hyperprolactinemic mice to study the role of PRL during lactation, because a single anterior pituitary graft increases serum homologous PRL to a level similar to that during lactation (1, 8). Estradiol replacement also might be physiological, judging from histological aspects of the uterus and vagina (27). Hence, PRL should induce insulin resistance in extramammary tissues with a synergistic estrogen action during lactation, although lactating animals will show no glucose intolerance overall, because the mammary gland is highly sensitive to insulin.

In conclusion, PRL decreases the glucose tolerance caused by insulin resistance in female tissues in the presence of estrogen. This coordination of PRL with estrogen will contribute to the homeorhesis of nutrients during lactation.

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