Absent or Delayed Preovulatory Luteinizing Hormone Surge in Experimental Diabetes Mellitus

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SUMMARY

The proestrus preovulatory luteinizing hormone (LH) surge was absent or delayed in more than 56% of untreated streptozotocin-diabetic rats. Absence of LH surge was associated with anovulation. Insulin treatment for 10–14 days restored the diminished surge and ovulation frequency. Pituitary LH release in response to exogenous gonadotropin-releasing hormone administration in diabetic rats was not different from controls. Impaired hypothalamic function may comprise the basis for the increased incidence of infertility in diabetes mellitus. DIABETES *33*:324–327, April 1984.

It has been generally accepted that diabetes mellitus (DM) in humans is associated with reproductive failure including delayed menarche, menstrual irregularities, and an increased incidence of infertility. Previous studies using alloxan-induced diabetic rats as a model also revealed delayed sexual maturity, an irregular estrous cycle, or a reduction in ovulation and the number of ova produced.[1] Recent studies demonstrated that immature female rats rendered diabetic by alloxan or streptozotocin showed an absent or diminished LH surge 2–3 days after exogenous administration of pregnant mare's serum with a failure in ovulation.[2,3] Since plasma estradiol levels were increased in both normal and DM rats after gonadotropin administration, abnormalities in LH release in DM have been hypothesized to be secondary to pituitary or hypothalamic dysfunction.[2,3] Kirchick et al.[4] reported a decreased pituitary LH response to exogenous administration of gonadotropin-releasing hormone (GnRH) in the immature alloxan-diabetic rats. Although a decreased LH and FSH release after exogenous administration of 100 μg of GnRH was reported in diabetic subjects,[5,6] another study revealed no alteration in gonadotropin release in response to the same maneuver.[7] Immature female rats differ from adults in that a continuing decrease in negative feedback sensitivity exists during prepubertal development, which results in a gradual rise in LH levels and subsequent production of sufficient estrogen to trigger the first preovulatory LH surge through positive feedback.[8] Thus, the preovulatory LH surge in adult rats may be more relevant to the investigation of the etiology of infertility in diabetes. The present study, therefore, was designed to characterize the preovulatory LH surge in regularly cycling adult rats with DM induced by streptozotocin.

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MATERIALS AND METHODS

Wistar female rats (Charles River) weighing 200–250 g were housed under constant temperature (24°C) and light (0600–1800 h) conditions. Only animals that demonstrated at least two 4-day estrous cycles, monitored by vaginal smear obtained between 0900 and 1000 h were used for the study. DM was induced by streptozotocin (Upjohn, Kalamazoo, Michigan) administration (65 mg/kg, i.p.) at 1700 h on the proestrus day. Control animals received vehicle alone (2 ml of 0.1 M sodium citrate buffer, pH 4.5). In some of these control animals, food was restricted to one third of the usual consumption (8 g/day). Urine volume and body weight were recorded daily. Glycosuria and ketonuria were monitored semiquantitatively by Keto-Diastix (Ames, Elkhart, Indiana). Since preliminary studies showed predominant estrous cycle regularity during the ensuing 6–7 days, the preovulatory LH surge was determined on the next presumptive proestrus day, 4 days after streptozotocin or vehicle administration by measuring LH levels in blood samples collected from the tail vein hourly from 1500 to 1800 h and at 2000 h, except as specified. In a second set of DM animals, insulin treatment (3 U/day, regular:lente 1:1 mixture, s.c.) was initiated 4 days after streptozotocin injection with one third at 0900 h and the remainder at 1700 h. On the first proestrus day after insulin therapy for 10–14 days, LH levels were determined

TABLE 1
Effect of DM or food restriction on plasma glucose, body weight, LH surge, and ovulation (mean ± SE)

	Number of animals	Plasma glucose* (mg/dl)	Change in body wt (%)	LH surge frequency† (%)	Ovulation rate (%)	Ova per ovulated animals
Control	12	123 ± 6	2.8 ± 0.5	12/12 (100 %)	8/8 (100 %)	12.5 ± 0.6
DM	18	539 ± 53§	−3.9 ± 1.2§	8/18 (44 %)§	5/9 (56 %)‡,‖	10.8 ± 0.4‡
DM with insulin	12	307 ± 69‖,¶	1.6 ± 3.2	10/12 (83 %)#	5/5 (100 %)	10.6 ± 1.3
Food restriction	7	120 ± 9¶	−8.5 ± 1.9§	7/7 (100 %)#	4/4 (100 %)	12.3 ± 0.3

*Measured by glucose-oxidase method without fasting at 1500 h.
†An LH peak greater than 200 ng/ml was regarded as a positive surge.
‡Animals in which a delayed time course for LH surge was determined from 1700 h to 2400 or 0200 h.
§$P < 0.01$ vs. control, ‖$P < 0.05$ vs. control, ¶$P < 0.01$ vs. DM, #$P < 0.05$ vs. DM.

as above. In selected animals, the number of ova was counted the next morning (estrus). To determine whether the abnormal LH release in DM is due to a failure of pituitary LH release in response to normal GnRH or to an impaired hypothalamic GnRH release per se, endogenous GnRH was blocked by pentobarbital injection (35 mg/kg, i.p.) at 1100 h on the next proestrus day in control and DM rats. Pituitary LH response to exogenous GnRH administration (500 ng/kg, i.v.) at 1200 and 1300 h was determined by serial LH determination through a catheter introduced into the right atrium via the jugular vein.[9]

Plasma LH levels were assayed by a double-antibody radioimmunoassay provided by NIAMDD and expressed as ng LH RP-1/ml.[10] Plasma estradiol concentrations were determined by a radioimmunoassay with a specific antibody (Miles) according to the method of R. L. Goodman,[11] using pooled plasma samples obtained at 1500 and 1600 h. Plasma glucose levels were measured without fasting by the glucose-oxidase method. All data were expressed as the mean ± SE and statistical significances were evaluated with Fisher's probability test, Student's *t* test or analysis of variance (one way).

RESULTS

As shown in Table 1, all control females showed an LH surge followed by ovulation, in which the peak appeared at 1600 h (N = 3), 1700 h (N = 5), 1800 h (N = 3), and 2000 h (N = 1). Streptozotocin administration resulted in marked hyperglycemia and body weight loss. When the LH surge was also monitored until 2000 h, only three of nine DM rats demonstrated the normal surge with peaks at 1800 h (N = 2) and 2000 h (N = 1). The overall pattern of LH surge (Figure 1A) was significantly lower in DM in comparison with controls. To determine whether the preovulatory LH surge in DM is simply delayed or totally absent, LH levels were monitored until 2400 or 0200 h, well beyond the normal range of 1600–2000 h, during which the peak was found in all controls. Individual results are summarized in Figure 1B. Five of nine rats showed a peak, three being delayed to 2000 h or later, followed by ovulation the next morning, with the number of ova shed the same as controls (Table 1). However, no surge was observed in the remaining four DM rats, in which anovulation was found the following morning despite a positive estrus smear. Thus, a normal or delayed LH surge was observed in eight of eighteen DM rats (Table 1). In insulin-treated diabetic animals, the frequency of the LH surge was similar to that of controls (Table 1), although the magnitude of the peak was lower than in controls (Figure 1A). Proestrus estradiol concentration measured in plasma samples obtained at 1500 and 1600 h in controls, DM, or insulin-treated DM was 15, 12, or 10 pg/ml, respectively. Since DM decreased body weight, one segment of this experiment included restriction of food to nondiabetic animals, and a subsequent analysis of the effects of the resultant decrease in body weight on the LH surge. Food restriction induced weight loss comparable with that found in DM (Table 1). However, the preovulatory LH surge occurred with peaks at 1700 h (N = 3), 1800 h (N = 2), and 2000 h (N = 2), resulting in a significantly higher LH surge in comparison with DM, but lower than that of controls (Figure 1A). Ovulation was also observed in the food-restricted group, with the number of ova identical to that of controls.

Pituitary LH response to exogenous GnRH administration was determined. It is evident from Figure 1C that exogenous GnRH administration induced the same LH response in DM and controls.

DISCUSSION

These results demonstrate that the LH surge occurs in normal, nondiabetic adult rats between 1600 and 2000 h. In adult diabetic rats, the surge is absent, delayed to, or beyond the latter part of the normal time period. A delayed LH surge in DM was associated with ovulation and a normal number of ova, while an absent LH surge was always associated with anovulation. Our results indicate that infertility in DM is related to a deficiency in LH release that may be attributed to impaired GnRH release, since there was a normal pituitary response to exogenous GnRH in diabetic rats. The results of our studies in adult diabetic rats differ from those conducted in immature rats.[4] The latter investigation suggested that the basis for infertility in DM was associated with pituitary insensitivity of LH to GnRH. However, it should be noted that immature rats have a different sensitivity of pituitary LH release to GnRH and estradiol in comparison with the adult rats used in this study.[8] It has been hypothesized that the ovaries in immature rats have a hyperresponsiveness to gonadotropin stimulus.[12] Second, the previous study employed a single i.p. injection of GnRH at a higher dosage (1–1.2 μg/100 g) without blockade of endogenous GnRH release.[4] It has been demonstrated that GnRH has a self-priming action upon gonadotropin release.[13] This phenomenon was also apparent in our study, judging from the marked LH rise in response to the second GnRH injection as compared with that obtained after the first injection. This

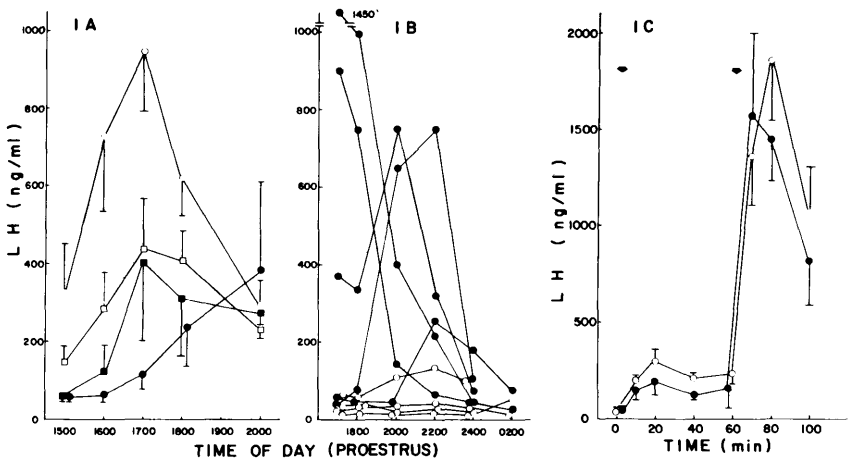

FIGURE 1. (A) The preovulatory LH surge in normal cycling rats (N = 12, ○), food-restricted rats (N = 7, □), and DM rats with (N = 12, ■) or without (N = 9, ●) insulin treatment. The LH surge was estimated by plotting plasma LH levels over time and measuring the area under the curve. Statistical significances evaluated by analysis of variance (one-way) are as follows. F value versus control: DM $F^1_{19} = 11.20$ ($P < 0.01$), DM with insulin, $F^1_{22} = 8.55$ ($P < 0.01$), food restriction, $F^1_{17} = 5.72$ ($P < 0.05$); F value versus DM: DM with insulin, $F^1_{19} = 0.24$ (NS), food restriction, $F^1_{19} = 7.68$ ($P < 0.05$). (B) Individual proestrus LH profiles in 9 DM rats measured from 1700 to 0200 h; ovulating (●) and anovulatory (○) rats. (C) LH responses to exogenous GnRH administration (500 ng/kg, i.v., twice as indicated by arrows) in control (N = 6, ○) and DM (N = 7, ●) rats. Plasma glucose levels measured at 1100 h were significantly higher in DM (560 ± 40 mg/dl) than in controls (118 ± 9 mg/dl). Difference in LH responses between two groups evaluated as above was not significant ($F^1_{11} = 0.60$).

pattern of LH response to GnRH is also dependent on estrogen levels because of fluctuations that occur throughout the cycle, with a maximum at proestrus.[13] Therefore, the adult proestrus rat is the most suitable model to evaluate the effect of DM on the preovulatory LH surge as well as the LH response to exogenous GnRH administration.

It has been generally accepted that loss of body weight causes a reduction in both LH release and response to GnRH in experimental animals,[14] as well as in patients with anorexia nervosa.[15] However, in this study, weight loss induced by food restriction did not affect the LH surge frequency, although the peak was lower in food-deprived animals than in controls. It is, therefore, quite possible that the abnormal LH surge in DM is not simply due to the loss of body weight, but more specifically related to DM itself. This view is supported by the fact that insulin treatment restored the diminished surge frequency, and that no LH surge was demonstrated in six spontaneously diabetic BB rats by 1700 or 2000 h.[16] Approximately 25% of matings of diabetic BB rats are nonproductive. Infertility could thus be explained, at least in part, by the fact that LH values were < 25 ng/ml at both test times. Another finding that suggests that hypothalamic dysfunction in DM may not be limited to deficient LH release is the observation that diminished thyrotropin-releasing hormone production occurs after thyroidectomy in DM.[17] Although precise mechanisms remain to be clarified by direct measurement or immunohistochemical staining of hypothalamic GnRH in diabetic animals, insulin deficiency per se or other metabolic changes induced by DM may be involved in hypothalamic dysfunction in DM. In fact, a reduced nuclear ^{3}H-estradiol concentration and a diminished number of nuclear estrogen receptors[18] were reported in the hypothalamus in untreated diabetic rats. Although our data on estrogen profiles in DM are preliminary, ovarian estrogen production seems to be sufficient to induce cyclic changes in vaginal cytology despite an abnormal LH surge.

In summary, it appears that the primary LH abnormality in the diabetic hypothalamic-pituitary-ovarian axis may reside in the hypothalamus. Whether the impaired hypothalamic GnRH release is primary or secondary to any alterations of ovarian steroids cannot be fully established on the basis of the present findings. However, whatever the mechanism, it seems evident that pituitary-hypothalamic LH dysfunction underlies the abnormalities in ovulation associated with insulin-dependent diabetes. The results of these studies form the basis for a more rational approach to the treatment of infertility in diabetes.

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