Serum Prolactin Response to Ether Stress in Diabetic Rats: Opiate System Contribution

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Abstract. **Diabetes in streptozotocin-treated rats is associated with alterations in various neuroendocrine systems, including endogenous opioids. These changes are suggested to be responsible for the significant reduction in serum prolactin (PRL) response to a brief restraint stress in diabetic male rats, as compared with normoglycemic controls. The present study examines serum PRL response to ether exposure in diabetic male rats. The animals' response to ether stress, which is known to be related to the opioid system, was examined twice in each rat: shortly after cannula insertion (Day 1), and seven days later. In order to evaluate the opiate system involvement, the experiment was repeated on Day 1 and 7 after surgery in a group of rats which were pretreated with naltrexone (Nalt), an opioid receptor antagonist. Opioid receptor sensitization was also performed by prior acute morphine administration on Day 7 after cannulation surgery. Following adaptation to the cannulation, no difference in serum PRL response to ether stress was found between diabetic and normoglycemic rats. However, on Day 1 after surgery, a significant difference was found between the diabetic and control groups: the normoglycemic (control) group exposed to ether responded to the surgical stress by augmented serum PRL levels. This response was not recorded in the diabetic rats. Opioid receptor blockade by Nalt administration 30 min before ether exposure eliminated this difference. Opioid receptor sensitization by morphine pretreatment facilitated PRL secretion in normoglycemic rats exposed to ether, while no effect could be distinguished in the diabetic group. It is therefore concluded that the streptozotocin-induced diabetic rats do not differ from normoglycemic ones in their ability to respond to acute ether stress by itself. However, enhanced PRL secretion induced by ether exposure under additional surgical stress, or by presensitization of the opioid receptors by morphine, is prevented in diabetic rats, probably due to diminished opioid receptor response.** [P.S.E.B.M. 1994, Vol 205]

Hormonal response to stress can be classified according to the source of stress and its duration. The response to acute stress is different from that to chronic stress. Chronic stress in streptozotocin-induced diabetic rats was shown to be accompanied by a reduced plasma β-endorphin level (1–3). Luteinizing hormone (LH) level in diabetic male rats was found to be attenuated, while the serum prolactin (PRL) level remained unchanged in comparison with normoglycemic rats (4, 5). Prolactin is known to be secreted in response to different kinds of stressors (6–8), but in diabetic rats, PRL response to stress, such as short periods of restraint, was significantly less compared with normoglycemic controls (5). The exposure of rats to ether for a few minutes is an accepted model for acute stress (9). Prolactin response to this stress has been suggested as being controlled by the dopaminergic and opioid systems (10–12). Moreover, ether stress has been shown to cause an increase in the concentration of plasma β-endorphin-like immunoreactivity, probably of pituitary origin (13).

The purpose of the present study was to evaluate the PRL response of diabetic male rats (chronic stress) to ether exposure (acute stress). Blood samples were

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collected twice from each rat on Days 1 and 7 after cannula implantation. Thus, with the first bleeding the animals were still under the influence of surgical stress, while on the second occasion (Day 7) they had already adapted to the cannula and responded only to the acute ether stress (14).

In order to evaluate the opioid system involvement in prolactin secretion, groups of diabetic and normoglycemic male rats were cannulated and subsequently treated either with the opioid receptor antagonist naltrexone (Nalt) or sensitized by morphine, prior to ether stress and blood collection (15, 16).

Materials and Methods

Animals and Treatments. Male Wistar rats, weighing 350–450 g, were housed in a temperature-controlled room under a 14 hr L/10 hr D lighting regime. Purina lab rat chow and tap water were provided *ad libitum*.

Diabetes was induced by ip injection of freshly-prepared streptozotocin solution at 40 mg/kg body wt (Calbiochem, La Jolla, CA), in a citrate buffer vehicle (100 m*M*, pH = 4.5). The diabetic state was verified by glucosuria and plasma glucose determination from the initial sampling after cannulation. Animals with serum glucose levels of less than 22.2 mmol/liter were excluded from this study. Control rats were injected with the vehicle only.

Naltrexone hydrochloride (Dupont, Switzerland), 1 mg/rat, was dissolved in 0.5 ml saline and injected iv. Morphine hydrochloride at a concentration of 5 mg/ml saline was given iv as a bolus at a dosage of 5 mg/kg body wt. Ether exposure was performed for 10 min between 2:00 and 3:00 PM, and the anaesthetized rats were bled.

Blood Collection. Blood for PRL analysis (0.4 ml/sample) was collected from an indwelling cannula inserted into the right cardiac atrium (14). Blood samples were centrifuged and the plasma was kept at −20°C. Blood cells were resuspended in saline and returned to the animals via the cannula after the next bleeding.

Glucose and PRL Assays. Glucose concentrations were measured using a Glucometer (Beckman, USA). Plasma PRL levels were determined by using a NIDDK and National Hormone and Pituitary Program (Baltimore, MD) radioimmunoassay kit. Rat PRL-RP-1 was used as a reference preparation. The lower limit of detection for the PRL assay was 2μg/l, while the intra- and inter-assay coefficients of variations were 9% and 15%, respectively.

Statistical analysis of results was performed using Wilcoxon's signed rank test and rank sum two sample test.

Experiment A. Ether exposure was performed on Days 1 and 7 after cannulation surgery in diabetic and normoglycemic rats.

Experiment B. The same procedure was repeated in additional groups of diabetic and normoglycemic rats in which opioid receptors had been blocked with 1 mg Nalt 30 min prior to the first bleeding.

Experiment C. Two additional groups of diabetic and normoglycemic rats were treated with morphine at 8:00 AM on Day 7 after cannulation, 6 hr before ether exposure.

In all groups (n = 9–11 each), the rats were exposed to ether between 2:00 and 3:00 PM. All experiments were carried out according to the Guidelines on the Use of Living Animals in Scientific Investigations.

Results

Glucose Levels. Glucose levels in the diabetic rats of Experiments A, B, and C were 30.1 ± 0.62, 32.4 ± 1.40, and 30.0 ± 1.13 m*M* (mean ± SE), respectively, while in normoglycemic rats the levels were 6.2 ± 0.17, 6.2 ± 0.25, and 6.0 ± 0.17 m*M*, respectively.

Prolactin Levels. *Experiment A.* On Day 1 after cannulation, plasma PRL levels, following a 5- and 10-min exposure to ether, in normoglycemic rats were higher than those of the diabetic animals (P = 0.024 and 0.002 at 5 and 10 min, respectively; Fig. 1). However, after 7 days of adaptation to the indwelling cannula implantation, no difference was found between the two groups. In both diabetic and normoglycemic rats, an elevation of PRL baseline level (0 time) was found on Day 1 following cannulation surgery, compared to Day 7 (P = 0.019 in the diabetic group, and P = 0.031 in the normoglycemic group), indicating the effect of acute surgical stress on both groups of rats shortly after cannula implantation.

Experiment B. In both groups, blocking opioid receptors with Nalt did not prevent PRL secretion in response to ether exposure ($P < 0.005$ and $P < 0.01$, comparing baseline levels and 5 and 10 min of ether exposure, respectively, on Days 1 and 7). However, Nalt did abolish the augmented PRL secretion on Day 1 after surgery in the normoglycemic rats (P = 0.0116, comparing groups in Experiments A and B for both 5- and 10-min ether exposure respectively; Fig. 1 and 2). Prolactin levels immediately before ether exposure in Nalt-treated rats (Experiment B) were lower compared with untreated rats (Experiment A) in diabetic (P = 0.0067 on Day 1; P = 0.0661 on Day 7), and normoglycemic rats (P = 0.0003 on Day 1, P = 0.0057 on Day 7).

Experiment C. After opioid receptor sensitization by morphine pretreatment on Day 7 postsurgery, a marked difference in PRL secretion was noted between diabetic and normoglycemic rats: morphine pretreatment in normoglycemic rats facilitated PRL secretion (baseline level [P = 0.005], as well as the response to ether [P = 0.001 at 5 min, P = 0.005 at 10 min] compared with normoglycemic rats with and

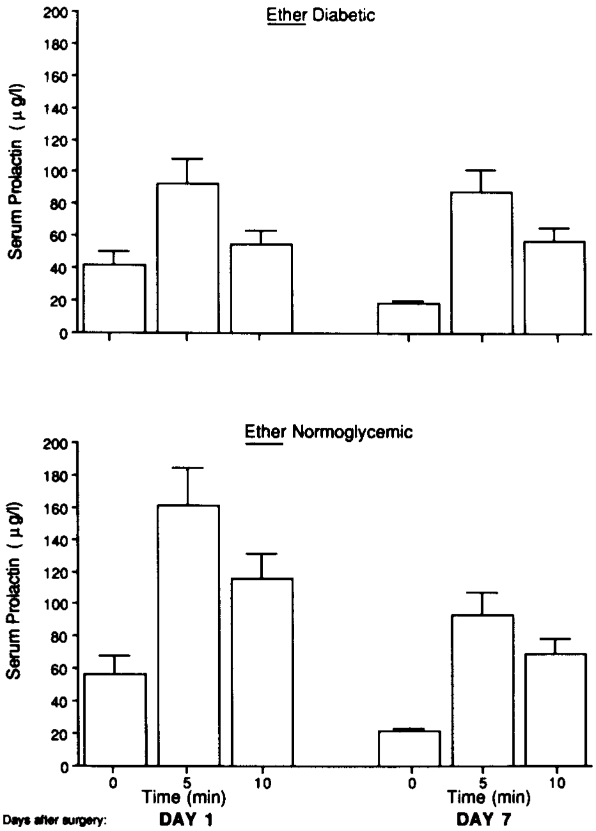

Figure 1. Serum prolactin levels before (0), 5, and 10 min following ether exposure. Upper panel, diabetic rats 1 and 7 days after surgery; lower panel, normoglycemic rats 1 and 7 days after surgery. Statistical differences are given in the text. Numbers are mean ± SEM of 9–11 rats for each group.

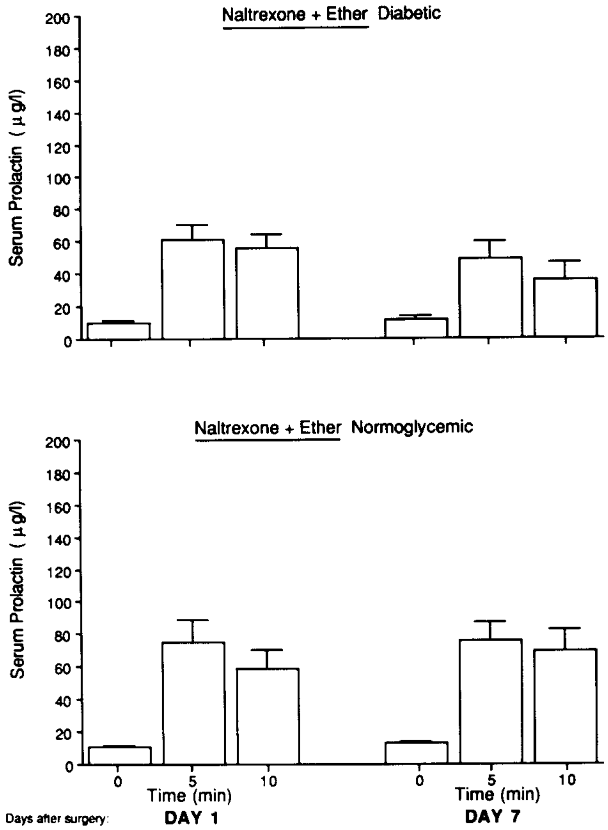

Figure 2. Prolactin response to ether stress in Naltrexone pretreated diabetic (upper panel) and normoglycemic (lower panel) male rats. Details as in Figure 1.

without morphine on Day 7), relative to untreated, 7-day-postoperation normoglycemic rats. In contrast, no difference could be noticed in the diabetic groups between the morphine-treated and untreated groups on Day 7 (Fig. 1 and 3).

Discussion

Rats with diabetes of four-weeks duration (chronic stress), have been shown to have a low serum β-endorphin level (3) and reduced response to opioid blockers (17). Thus, it was interesting to evaluate the PRL secretion as a response to ether (acute stress), which is known to be related to the opioid system control. It has been reported that various types of surgery also induce opioid secretion (18). In addition, hypersecretion of PRL has already been shown to occur on Day 1 after cannulation surgery (16). In the present study, by using the diabetic rat model, a clear distinction was shown between the surgical stress and ether stress. Surgery did indeed cause serum PRL elevation (baseline levels were high on Day 1, compared with Day 7 after cannulation) in the diabetic as well as the normoglycemic rats. However, the hypersecretion of PRL in response to ether was augmented by the surgical stress in the normoglycemic rats only. This increase could be abolished by Nalt pretreatment, indicating opioid system involvement. It is suggested that the attenuation of the opioid-system activity in diabetic rats is responsible for the inability of these rats to react to the surgical stress by enhanced PRL secretion following ether exposure.

By sensitizing the opioid system with morphine pretreatment (5 mg/kg body wt, 6 hr prior to ether exposure) the difference between diabetic and normoglycemic rats became evident: unstressed, normoglycemic rats on Day 7 after cannulation (Fig. 3) responded with markedly elevated PRL levels (both prior to and following ether exposure) compared with the rats which were not pretreated with morphine (Fig. 1). No such PRL elevation was seen in the morphine pretreated diabetic rats on Day 7 after cannulation.

Since after 7 days of adaptation to the cannulation no difference in response to ether stress was found between diabetic and normoglycemic rats (Fig. 1), or between Nalt pretreated and untreated rats, (except for diabetic rats, at 10 min only), this would appear to indicate that the opioid system has a minimal or nonexistent role in the control of PRL secretion as a response to ether stress. It is suggested that reduced dopamine release into the hypophyseal portal blood is responsible for the increase in PRL concentration.

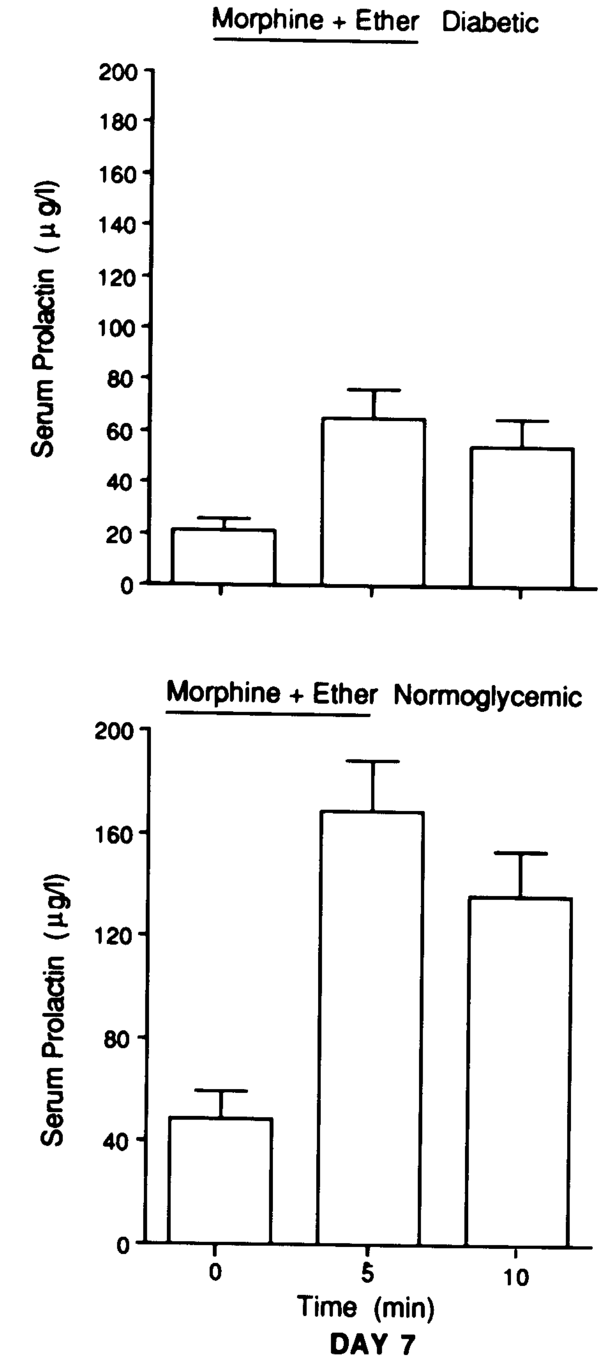

Figure 3. Morphine pretreated diabetic (upper panel) and normoglycemic (lower panel) male rats 7 days after surgery. Details as in Figure 1.

Diabetic rats are unable to respond to morphine by enhancement of PRL secretion as a response to ether stress. This might be due to a hyperglycemia-induced decrease in the number of receptors that are available to bind morphine, or the alteration in their conformation, rather than opioid deficiency. This is in correlation with the finding that antinociceptive potency of morphine was significantly decreased in rats with streptozotocin-induced diabetes (19).

In an earlier study (20), male rats given a single injection of morphine sulfate demonstrated significantly higher circulating PRL levels for 60 min, with a return to baseline levels thereafter. Thus, the high PRL levels in the normoglycemic rats in the present study (Experiment C) at 0 and 6 hr after morphine injection, in comparison with untreated rats (Experiment A) could be a function of the extreme sensitivity of the animals to nonspecific stimuli due to the opioid system hypersensitivity.

It is concluded that the diabetic rat does not differ from the normoglycemic one in its pattern and magnitude of PRL response to acute ether stress (7 days of adaptation without additional stress). However, it does lack the normal capacity to mobilize the opioid system. It is suggested that the difference in PRL levels between diabetic and normoglycemic rats under ether stress becomes evident when additional stressors, which facilitate PRL secretion by sensitizing the opioid receptors, accompany the ether stress, as was verified by the Nalt and morphine pretreatments.

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