Diabetes Induced by Streptozocin Results in a Decrease in Immunoreactive Beta-Endorphin Levels in the Pituitary and Hypothalamus of Female Rats

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SUMMARY
Immunoreactive beta-endorphin (IR-BE) was measured by radioimmunoassay in the anterior pituitary (AP), neurointermediate lobe of the pituitary (NIL), and hypothalamus of female rats 4 wk after being made diabetic by a single injection of streptozocin (STZ). STZ-induced diabetes resulted in a significant reduction in the content and concentration of IR-BE in the AP and the content of IR-BE in the hypothalamus. Total hypothalamic protein was also significantly diminished. IR-BE levels in the NIL were unchanged. Column chromatography indicated that the reduction in IR-BE in the AP of the diabetic female rats represented a decrease in peptides that co-eluted with beta-endorphin and beta-lipotropin. In the hypothalamus, the reduction in IR-BE was represented solely by a decrease in a peptide co-eluting with beta-endorphin. Beta-lipotropin was not detectable in the hypothalami of control or diabetic female rats. These results suggest that, in the rat, diabetes may produce alterations in the mechanism(s) that regulate endogenous opiate levels in the pituitary and hypothalamus. DIABETES 1985; 34:1104-107.

Beta-endorphin has been localized in the pancreas1 and both beta-endorphin2 and morphine3 have been shown to be capable of directly stimulating the release of insulin and glucagon from the pancreas. Furthermore, systemic administration of beta-endorphin resulted in an increase in plasma levels of insulin and glucagon.4 These findings suggest that endogenous opiates may participate in the regulation of glucose homeostasis. However, this interpretation of these observations is contradicted by reports that insulin-induced hypoglycemia produced—an increase in plasma levels of beta-endorphin.5,6 Thus, the role of endogenous opiates in the mediation of steady-state glucose levels awaits further clarification. Although beta-endorphin is found in the pancreas, the major sites in the body of beta-endorphin synthesis and release are the pituitary7,8 and hypothalamus.9,10 Recent evidence indicated that diabetes, as induced with STZ, resulted in a significant alteration in both pituitary and hypothalamic neuropeptide levels.11-13

At the present time, little is known about how diabetes affects the synthesis and release of beta-endorphin by the hypothalamo-hypophyseal unit. It was the goal of the present study to determine the effects of diabetes induced with STZ on pituitary and hypothalamic beta-endorphin levels in the rat. The results of this investigation should provide additional insight into the effect of diabetes on neuroendocrine function.

MATERIALS AND METHODS
Animals. Female Sprague-Dawley rats (200–250 g) were obtained from Hilltop Farms (Scottsdale, Pennsylvania). Animals were maintained in a temperature-controlled room (22 ± 2°C) and kept on a 14:10 h light:dark cycle (lights on at 0500 h). Food (Ziegler Diet, Buckshire Farms, Landsdale, Pennsylvania) and water were available ad libitum.

Induction of diabetes. Animals demonstrating four consecutive 4-day vaginal estrous cycles were used in the present study. Animals were injected with 50 mg/kg of streptozocin (STZ, Sigma Chemical Co., St. Louis, Missouri) dissolved in 1% citrate buffer. Control animals were injected with buffer alone. Induction of diabetes was confirmed after 1 wk by the measurement of urine glucose levels using Ames Clinitest (Ames Division, Miles Laboratories, Elkhart, Indiana). At the end of 4 wk, all animals were killed. Blood glucose concentrations were determined using a glucose-oxidase method (Sclavo Inc., Wayne, New Jersey) and only animals with glucose levels of 400 mg/dl or greater were considered to be diabetic and included in the study.

Collection of pituitaries. Animals were decapitated and pituitaries were removed and separated into the anterior pi-
ultitary (AP) and neurointermediate lobe of the pituitary (NIL). Each pituitary portion was placed in 12 × 75-mm polystyrene test tubes, to which 2 ml of phosphate buffer (0.02 M NaPO₄, 0.15 M NaCl, 10 mM EDTA, and 0.01% sodium azide, pH 7.5) containing 1 mM N-ethylmaleimide had been added. Pituitary portions were then homogenized and frozen at −20°C. Pituitary content was measured by the technique of Lowry et al.

Collection of hypothalami. At the time of decapitation, the whole brain was removed and quickly frozen on dry ice. Each brain was then partially thawed and the hypothalamus dissected. Cuts were made 3 mm anterior to the optic chiasm and rostral to the mamillary bodies. Sections were bordered laterally by the lateral hypothalamic sulci and dorsally by a cut at the top of the third ventricle. The sections included the organum vasculosum of the laminae terminalis, the preoptic area, the supraoptic nucleus, the suprachiasmatic nucleus, the arcuate nucleus, the ventromedial nucleus, the infundibular stalk, the arcuate nucleus, the ventromedial nucleus, and the dorsomedial nucleus. Tissues were homogenized in buffer containing 1 mM N-ethylmaleimide and stored in 12 × 75-mm polystyrene tubes at −20°C. Hypothalamic protein content was measured by the technique of Lowry et al.

Radioimmunoassay of beta-endorphin. The radioimmunoassay for immunoreactive beta-endorphin (IR-βE) has been described in detail elsewhere, along with its suitability to measure IR-βE in the rat. In our hands, the antibody to beta-endorphin (generously supplied by Dr. S. S. C. Yen, University of California, San Diego, California) demonstrated a complete cross-reactivity with beta-lipotropin on a molar basis. Increasing volumes of plasma, pituitary and hypothalamic lysates were found to be parallel to the standard curve for camel (ovine, bovine, rat) beta-endorphin (Peninsula Laboratories, San Carlos, California). The intra- and interassay coefficients of variation were 8% and 13%, respectively. All samples of a given tissue (i.e., pituitary, hypothalamic lysates or column eluates) were assayed together.

Gel chromatography. Samples were chromatographed in a 1.6 × 70-cm glass column packed with Sephadex G-50 medium. Three milliliters of either pooled pituitary or hypothalamic lysate (diluted in assay buffer) were applied to the column. The column was eluted with 0.1 N acetic acid, 0.05% bovine serum albumin, and 0.02% sodium azide. Fractions of 1.5 ml were collected. Elution profiles were obtained by determining the immunoreactivity of each fraction using the beta-endorphin radioimmunoassay, which recognized beta-endorphin and beta-lipotropin equally. The elution profiles of the samples were compared with those obtained for either camel (ovine, bovine, rat) beta-endorphin or human beta-lipotropin (generously provided by the National Pituitary Agency, NIH). Immunoreactivity producing an elution profile similar to that of camel beta-endorphin was considered to be beta-endorphin. Similarly, immunoreactivity producing an elution profile identical to that of beta-lipotropin was considered to be beta-lipotropin.

Statistical analysis. Statistical analysis of the data was performed using Student’s t-test. A significance level of P < 0.05 was chosen.

RESULTS

Characterization of diabetes. Before time of killing, all STZ-treated rats exhibited glycosuria at a level of 2+ or 3+ (Ames Clinitest), while all control animals were negative for urine glucose. Blood glucose levels in the rats made diabetic with STZ were significantly increased over those values obtained in control animals (564.2 ± 26.9 and 119.6 ± 3.8, respectively; P < 0.05). Body weights were reduced slightly in the diabetic group (10%) as compared with controls; however, this difference was not significant.

Pituitary and hypothalamic IR-βE. The content and concentration of IR-βE was significantly reduced (P < 0.05) in the AP of diabetic versus control female rats (Table 1), while total protein in the AP was similar in both groups (Table 1).

<table>
<thead>
<tr>
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<th>Control rats</th>
<th>Diabetic rats</th>
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<tbody>
<tr>
<td>AP Content (μg/gland)</td>
<td>3.09 ± 0.13</td>
<td>2.57 ± 0.14†</td>
</tr>
<tr>
<td>Concentration (μg/mg protein)</td>
<td>1.58 ± 0.09</td>
<td>1.12 ± 0.06†</td>
</tr>
<tr>
<td>Total protein (mg)</td>
<td>2.00 ± 0.11</td>
<td>1.86 ± 0.14</td>
</tr>
<tr>
<td>NIL Content (μg/gland)</td>
<td>14.72 ± 2.13</td>
<td>15.61 ± 1.49</td>
</tr>
<tr>
<td>Concentration (μg/mg protein)</td>
<td>10.37 ± 1.15</td>
<td>10.39 ± 1.04</td>
</tr>
<tr>
<td>Total protein (mg)</td>
<td>1.33 ± 0.09</td>
<td>1.37 ± 0.08</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± SEM of the number of determinations indicated.
†Significantly different (P < 0.05) from control female rats.
of diabetic animals, beta-endorphin comprised 36% of the total IR-BE detected in the gland, whereas in control animals, 28% of the total IR-BE detected was beta-endorphin.

Beta-lipotropin was not detected in the hypothalami of animals from either group. Thus, the reduction in hypothalamic IR-BE observed in the diabetic female rats represented a decrease in beta-endorphin and not beta-lipotropin (data not shown). As in the hypothalami, beta-lipotropin was not found to be present in the NIL of control or treated animals.

**DISCUSSION**

The induction of diabetes in female rats by treatment with STZ resulted in a reduction in the content and concentration of beta-endorphin in the AP as well as a decrease in the content of beta-endorphin in the hypothalamus. A decrease in total hypothalamic protein was also observed in the diabetic animals. Neither the content nor the concentration of beta-endorphin in the NIL was affected.

The findings obtained in the present study are in contrast with those reported by Taylor et al., who found that, in male rats, IR-BE levels in the AP, NIL, and hypothalamus were unaffected by STZ-induced diabetes. These differences may be the result of the length of time that the animals were diabetic before time of killing, as well as the sex of the animals used in each study. In the present investigation, animals were killed 4 wk after treatment with STZ, whereas in the report by Taylor et al., the animals were killed 1 wk after treatment. The findings of the present study would suggest that a period of hyperglycemia longer than 1 wk is required before alterations in pituitary and hypothalamic beta-endorphin levels are detectable. In addition, gonadal steroids appear to influence pituitary and hypothalamic levels of beta-endorphin in the rat. It is possible that the effect of diabetes on beta-endorphin levels may differ in males as compared with female animals.

It may be speculated that the decrease in beta-endorphin levels in the AP of diabetic female rats may result in part from an increased release of the endogenous opiate with the onset of diabetes. Supporting such a hypothesis is the report of decreased dopamine metabolism in the brain of diabetic rats. Dopamine has been shown to inhibit beta-endorphin release from the pituitary, thus, a reduction in hypothalamic dopamine might also promote the release of beta-endorphin by the AP.

The decrease in beta-endorphin levels in the AP may also result from several other factors. Protein synthesis is diminished when insulin is either reduced or absent. It has been shown that, like beta-endorphin, pituitary concentrations of growth hormone and thyroid-stimulating hormone are decreased in diabetic animals. It is possible that a decrease in beta-endorphin synthesis may contribute to the reduction in pituitary beta-endorphin levels in diabetic rats, especially if beta-endorphin release from the AP is elevated, even if only at the onset of diabetes. Such a transient release of beta-endorphin coupled with a decrease in endogenous opioid activity in diabetic female rats, was the observation made in the present investigation of a significant reduction in total hypothalamic protein in these diabetic rats.

It is well-documented that the endogenous opioid peptides are antinociceptive in action in animals and humans. The results of the present investigation indicate that, in the AP and the hypothalamus of diabetic female rats, levels of the endogenous opiate beta-endorphin, are diminished. Supporting this finding, and further suggesting a decrease in endogenous opioid activity in diabetic female rats, was the observation of a decrease in the pain threshold in diabetic female rats as indicated by a significant reduction in paw-lick latency in response to hot-plate analgesia testing (Vasilenko and Lewis, unpublished observation).

Thus, the results of this study indicate that pituitary and hypothalamic levels of beta-endorphin are diminished in diabetic female rats. The reductions may reflect diabetes-induced alterations in the metabolism of beta-endorphin in these areas. Although the relationship between endogenous opiates and glucose homeostasis remains to be clearly established, diabetes does appear to influence the factors responsible for the maintenance of beta-endorphin levels in the hypothalamic-pituitary axis.

**ACKNOWLEDGMENTS**

This study was supported by grants to L.J.F. and P.V. from the University of Medicine and Dentistry of the New Jersey Foundation.

**REFERENCES**


