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Effect of Hypoglycemic Stress on the Pars Intermedia of the Mouse Pituitary Gland: An Ultrastructural Analysis

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ABSTRACT—The present study was made to clarify the relationship between functions of the pars intermedia of the mouse pituitary gland and hypoglycemic stress. Morphometrical analysis of the ultrastructures of the pars intermedia cells showed (1) a rise in the percentage volume of rough endoplasmic reticulum (r-ER) indicative of an increase in protein synthesis, (2) an increase in the number of Golgi granules per unit Golgi area showing an induction of granule-forming activity and (3) a decrease in the numerical density of secretory granules reflecting a release of the secretory granules. These findings suggest that hypoglycemic stress induced by daily treatment with insulin or restriction of food intake was able to elicit heightened secretory activity of the pars intermedia cells of the mouse pituitary gland. However, acute hypoglycemic stress induced by food deprivation did not cytologically affect the pars intermedia. These observations suggest that repeated hypoglycemic stress, rather than acute hypoglycemic stress, may be a natural physiological stimulus of the pars intermedia of the mouse pituitary gland.

INTRODUCTION

The pars intermedia of the pituitary gland produces and releases pro-opiomelanocortin (POMC)-derived peptides including β -endorphin and α -melanocyte stimulating hormone (α -MSH) [10, 12]. It is now widely accepted that the inhibitory control of POMC-derived peptides secretion from pars intermedia cells is mediated by dopaminergic neurons originating in the arcuate nucleus of the hypothalamus [2, 6].

In mammals, many investigators have reported on the physiological roles of POMC-derived peptides produced by the pars intermedia [13, 14]. However, the main physiological role of the pars intermedia is still poorly understood.

Several earlier studies have shown hyperfunction of the pars intermedia during the fetal and postnatal periods [11, 16, 20]. Our previous studies showed that immaturity of the hypothalamic inhibitory control mechanism on the pars intermedia resulted in marked hypersecretion of various hormones [9]. There is evidence that most newborn mammals, including humans, are susceptible to neonatal hypoglycemia shortly after birth [4], and that there is a parallelism between blood glucose levels and secretory activity of the pars intermedia [1, 24]. However, little is known about the effect of stimuli and conditions, in particular the effect of hypoglycemic stress, on secretory activity of the pars intermedia of the mouse pituitary. Therefore, the present study was designed to investigate the effect of hypoglycemic stress induced by repeated insulin injection, and hypoglycemic stress induced by repeated restriction of food intake on the secretory function of pars intermedia of the mouse pituitary.

MATERIALS AND METHODS

Animals

Male mice (6–7 weeks of age) of the JCL/ICR strain (CLEA Japan, Inc.) were used in our experiments. Animals were housed under controlled conditions in a 12 hr light and 12 hr dark cycle. Mouse chow and water were made available ad libitum.

Hypoglycemia induced by insulin injection

Porcine insulin (NOVO, Nordisk, Denmark) was dissolved in phosphate-buffered saline (PBS) and injected intraperitoneally into seven intact male mice at a dose of 5 U/kg daily for 3 days. Control mice were given vehicle only.

Hypoglycemia induced by repeated restricted food intake

For 3 days, seven mice were given normal mouse food (CA-1, CLEA Japan, Inc.) for only 1 hr per day.

Hypoglycemia induced by food-deprivation

For 3 days, seven mice were given only water without food as a model for acute hypoglycemic stress.

Hyperglycemia induced by streptozotocin injection

Streptozotocin (Wako Pure Chemical Industries, Ltd.) was dissolved in 0.05 M citrate buffer and injected intraperitoneally into seven intact male mice at a dose of 50 mg/kg. Control mice were given vehicle only.

Blood glucose analysis

Animals were sacrificed by decapitation, blood was immediately collected and blood glucose was determined by the glucose oxidase method (Glucoscan 2000, EIKEN Kagaku Inc.).

Ultrastructural morphometry of the pars intermedia

The animals were sacrificed by decapitation, and their pituitaries were fixed with phosphate-buffered 3% glutaraldehyde and postfixed with 1% OsO_4 (pH 7.4) for 1 hr, respectively. After dehydration, the specimens were embedded in Araldite-Epon mixture and sections

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were cut parallel to the sagittal plane of the gland. Contrast in thin sections was obtained with uranyl acetate and lead citrate, and electron micrographs were taken at an original magnification of $\times 3,400$ and optically enlarged to a final magnification of $\times 10,000$. From these electron micrographs, 50–60 pars intermedia cells from each group were chosen at random, and morphological analyses were made on the percentage volume of the rough endoplasmic reticulum (r-ER) [7], and the numerical density of both secretory granules and Golgi granules [25]. The determinations of the above-mentioned parameters were mathematically corrected in such a way that each determined cell size within each experimental group was multiplied by the ratio of the mean cell size of the control to the mean cell size of the same experimental group, and the final determinations were recalculated for comparison. The number of Golgi granules was estimated in the same way on the basis of changes in the mean Golgi area of the control group to that of the experimental groups. Statistical analyses were based on the Student's *t*-test.

RESULTS

Effects of hypoglycemia induced by repeated insulin injection on the pars intermedia of the pituitary gland

Repeated insulin injection induced hypoglycemia (Fig. 1). Hypoglycemic stress induced by daily treatment with

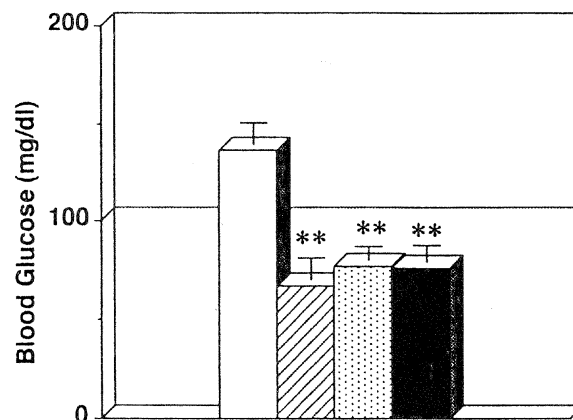


Fig. 1. Blood glucose levels of control mice (□) and hypoglycemic mice induced by insulin-treatment (▨), restricted food intake (▤) and food-deprivation (■). Data represent mean \pm SE. **, significantly different from control ($p < 0.01$).

insulin caused marked hyper-activity in cells of the pars intermedia. In the pars intermedia cells of mice treated with insulin for 3 consecutive days, there was a significant increase both in the percentage cytoplasmic volume of the r-ER and in the numerical density of Golgi granules, and there was a

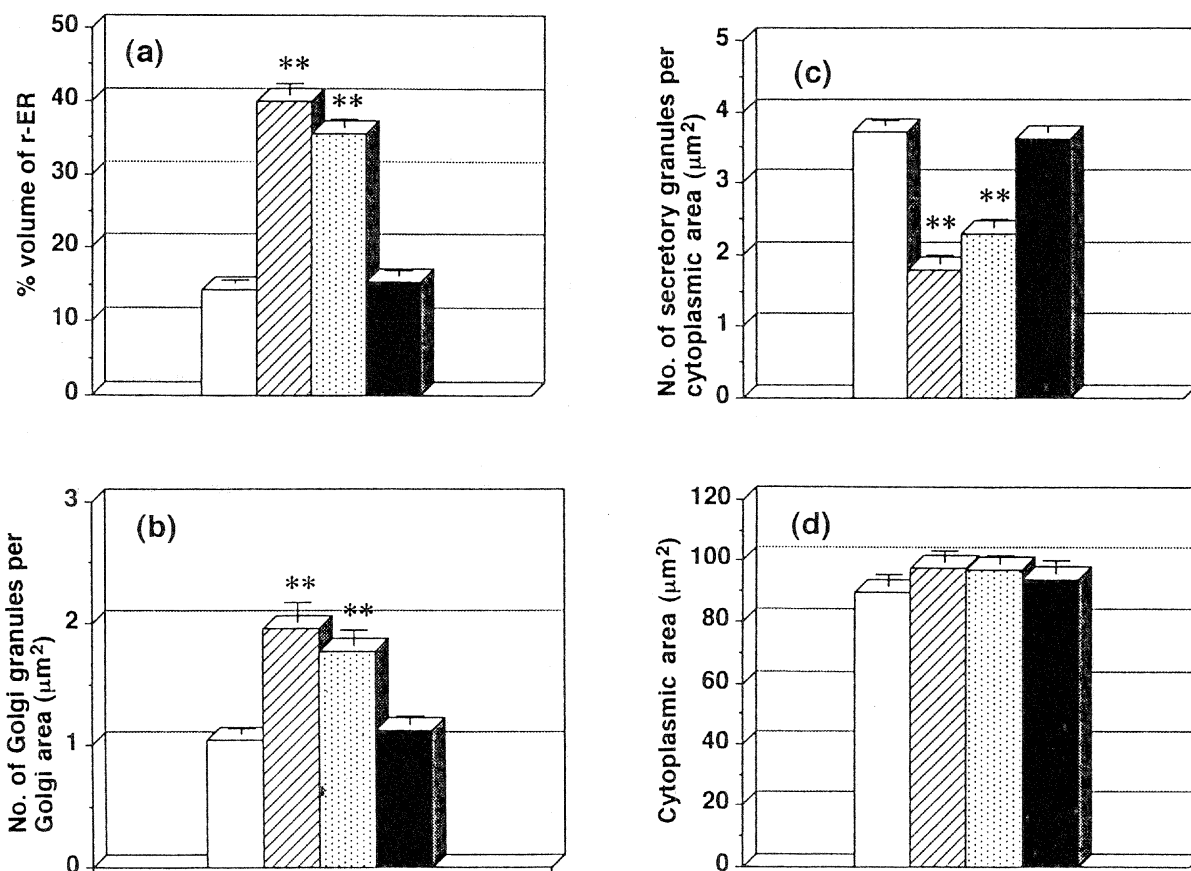


Fig. 2. The ultrastructural morphometrical parameters in the pars intermedia cells of control mice (□), and hypoglycemic mice induced by insulin-treatment (▨), restricted food intake (▤) and food-deprivation (■). (a) Percentage of the cytoplasm occupied by the rough endoplasmic reticulum. (b) The numerical density of immature Golgi granules per Golgi area (μm^2). (c) The numerical density of secretory granules per cytoplasmic area (μm^2). (d) Cytoplasmic area within the pars intermedia cells (μm^2). Data represent mean \pm SE. **, significantly different from control ($p < 0.01$).

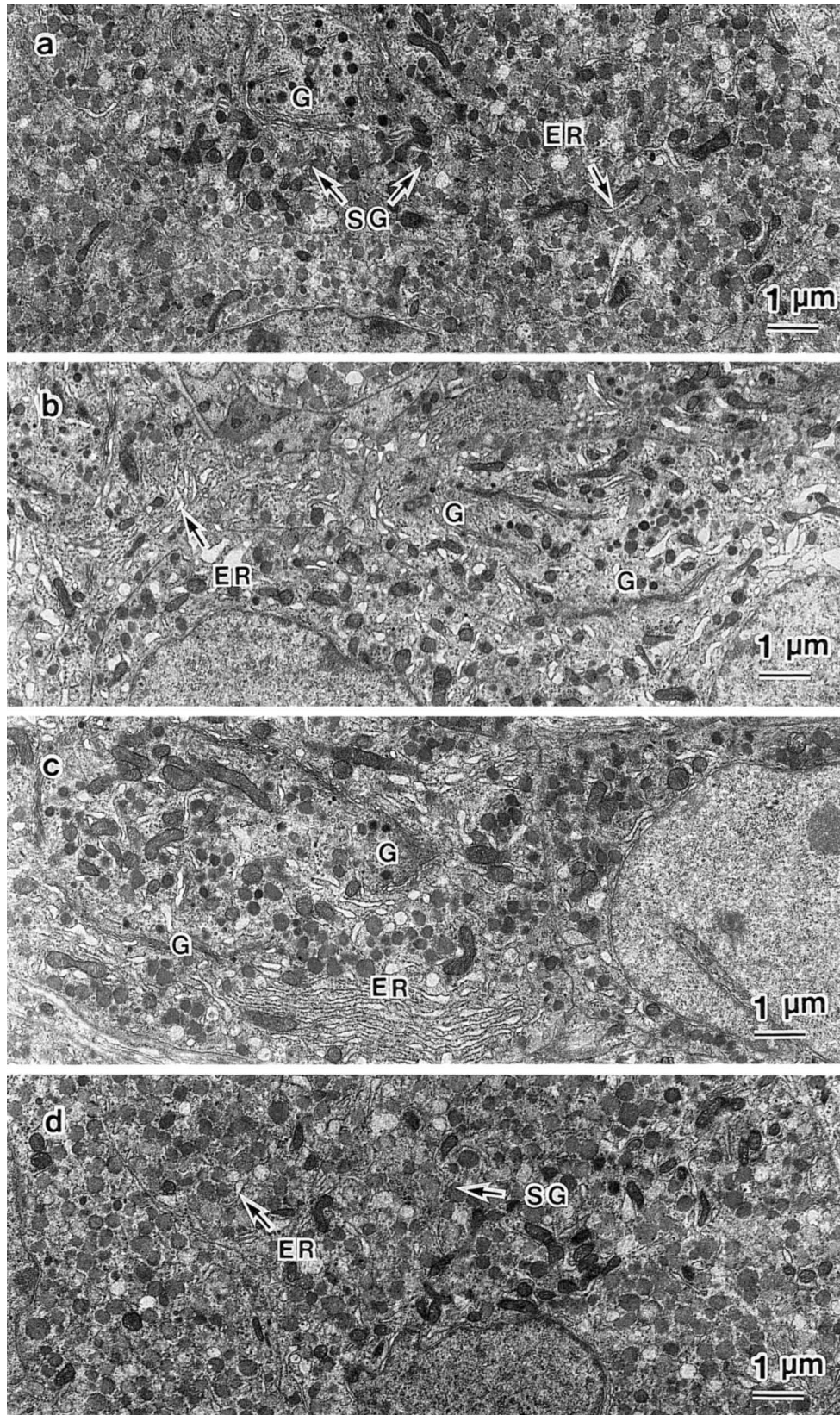


FIG. 3. Electronmicrographs of pars intermedia cells. (a) Pars intermedia cells of a control mouse. Note numerous secretory granules (SG), sparse rough endoplasmic reticulum (ER) with short tubular cisternae, and less prominent Golgi apparatus (G). $\times 7,480$. (b) Pars intermedia cells after 3 days of daily insulin-treatment. Note fewer secretory granules, well developed rough endoplasmic reticulum (ER) and prominent Golgi apparatus (G). $\times 7,480$. (c) Pars intermedia cells after 3 days of restricted food intake. Note fewer secretory granules, well developed rough endoplasmic reticulum (ER) and prominent Golgi apparatus (G). $\times 7,480$. (d) Pars intermedia cells after 3 days of streptozotocin-treatment. Note numerous secretory granules (SG) and sparse rough endoplasmic reticulum (ER) similar to those in control pars intermedia cells. $\times 7,480$.

significant decrease in the numerical density of secretory granules in the cytoplasm as compared to those of control mice (Fig. 2a, b, c, Fig. 3b). There was no difference in the cytoplasmic area of the pars intermedia cells between insulin-treated and control mice (Fig. 2d).

Effects of hypoglycemia induced by repeated restricted food-intake on the pars intermedia of the pituitary gland

If daily repeated hypoglycemia is responsible for the activation of intermedia cells, repeated food-restriction should cause a similar increase in activity of the gland. Repeated restricted food-intake also induced hypoglycemia (Fig. 1). Our results indicated significant differences in the three ultrastructural parameters studied on day 3 of the treatment schedule (Fig. 2a, b, c, Fig. 3c). However, there was no difference in the cytoplasmic area of the pars intermedia cells between food intake-restricted and control mice (Fig. 2d).

Effect of hypoglycemia induced by food-deprivation on the pars intermedia of the pituitary gland

The food-deprived mice exhibited significantly decreased plasma glucose levels similar to those observed in insulin-treated and food intake-restricted mice (Fig. 1). Although plasma glucose levels appeared to be at a level similar to those observed in the insulin-treated and food intake-restricted mice, the three ultrastructural parameters under observation were found to be the same as those of the control group after 3 days of continuous food-deprivation (Fig. 2a, b, c). Again, there was no difference in the cytoplasmic area of the pars intermedia cells between food intake-deprived and control mice (Fig. 2d).

Effect of streptozotocin-induced hyperglycemia on the pars intermedia of the pituitary gland

Effect of hyperglycemic stress on the pars intermedia cells was also examined by electron microscopy. The ultrastructural morphological characteristics of the pars intermedia cells of streptozotocin-treated mice (blood glucose levels; 286 ± 17 mg/dl) were not different from those observed in the pars intermedia cells of control mice (Fig. 3d).

DISCUSSION

The neonatal period is a critical time for survival in all mammals and neonatal animals require an ability to cope with severe stresses, such as hypothermia and hypoglycemia [4]. Many investigators demonstrate hypersecretion of POMC-related peptides from the pars intermedia during the neonatal period [11, 16, 20].

In this study, we demonstrated that hypoglycemic stress induced by repeated insulin treatment was able to elicit heightened secretory activity in the pars intermedia cells of the mouse pituitary gland. Morphometric examination by electron microscopy revealed apparent cytological signs of hyperfunction in pars intermedia cells. These included (1) a

rise in the percentage volume of the r-ER indicative of stimulated protein synthesis, (2) an increase in the number of Golgi granules per unit Golgi area showing an induction of granule-forming activity and (3) a decrease in the numerical density of secretory granules reflecting release of the secretory granules. Furthermore, repeated hypoglycemic stress induced by restriction of food intake also augmented secretory activity of the pars intermedia, as evidenced by a significant increase in the percentage volumes of the r-ER and Golgi granules accompanied by a decrease in the number of secretory granules. Repeated daily hypoglycemic stress may physiologically attenuate dopaminergic inhibitory control on the pars intermedia resulting in the enhanced secretory activity of the pars intermedia cells.

Previous studies demonstrated that acute insulin-induced hypoglycemia augments the POMC m-RNA levels in corticotrophic cells (ACTH cells) of the anterior pituitary, whereas it does not affect the POMC m-RNA levels in pars intermedia cells [23]. This activation of ACTH cells was mediated by an increase in corticotropin-releasing factor (CRF) levels of the hypothalamus [18]. Although food deprivation decreased blood glucose levels [3] and increased the concentration of CRF in the intermediate lobe of the pituitary [19], acute hypoglycemic stress induced by food deprivation did not affect pars intermedia cells in the present study. It is still unclear why acute hypoglycemic stress failed to change the ultrastructure of the pars intermedia cells. Since secretory activity of the pars intermedia of the adult mouse is strongly inhibited by dopaminergic neurons originating from the hypothalamus, repeated hypoglycemic stress, rather than acute hypoglycemic stress, might reduce dopamine turnover in the hypothalamus, which in turn may result in a diminished inhibitory effect on the secretory activity of the pars intermedia [17].

On the other hand, streptozotocin- or alloxan-induced diabetes reduces immunoreactive β -endorphin concentrations in pars intermedia cells of the rat pituitary [5, 21]. Although streptozotocin treatment significantly increased the blood glucose levels (286 ± 17 mg/dl) compared to the control levels (137 ± 10 mg/dl), we could not observe any morphological changes in the pars intermedia cells of the treated mice as compared to the pars intermedia cells of control mice. As to the possible mechanism for the reduction of immunoreactive β -endorphin contents in the pars intermedia, streptozotocin may enhance dopamine turnover in the hypothalamus [17] and an increase in dopaminergic input in the pituitary may in turn reduce the synthesis of immunoreactive β -endorphin in the pars intermedia. These results suggest that hyperglycemia may be a condition inhibitory to the activity of the pars intermedia.

Our previous morphological study demonstrated hyperfunction of pars intermedia cells in the new-born mice [9]. As MSH is related to steroidogenesis during the prenatal and neonatal periods [15] and to fetal growth [20], the pars intermedia may play an important role during these periods. Previously, we reported that copious drinking of water elic-

ited hypertrophy of pars intermedia cells. This result suggests that the pars intermedia may play a role in hydro-mineral regulation in the mouse [8]. MSH and endorphin receptors are widely distributed in mammalian tissues [22]. Therefore POMC-related peptides produced by cells of the pars intermedia may have wide-spread effects on body tissues. Thus, these results suggest that the peptides produced from the pars intermedia may be multi-functional in mammals, especially during the prenatal and neonatal periods, and may play a role in glucose homeostasis for survival under hypoglycemic stress.

In this study, for the first time, we morphologically showed that the pars intermedia of the mouse pituitary gland actively reacted to repeated hypoglycemic stress induced by insulin-treatment or restricted food intake. These results suggest that repeated hypoglycemic stress rather than acute one may be a natural physiological inducer of the function of the pars intermedia. Therefore, it is probable that many undefined multiregulatory mechanisms may act on the pars intermedia. We are now studying the control mechanisms of dopaminergic neurons on cells of the pars intermedia using primary culture methods.

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