EFFECT OF INSULIN-INDUCED HYPOGLYCEMIA AND GLUCOSE-INSULIN INFUSION ON BRAIN GLUCAGON-LIKE MATERIALS IN THE DOG

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ABSTRACT

The concentration of glucagon-like materials, *i.e.*, glucagon immunoreactivity (GI) and glucagon-like immunoreactivity (GLI), in the canine brain was measured by radioimmunoassay before and after injection of insulin or concomitant infusion of glucose and insulin. The amount of GI in the thalamus-hypothalamus was increased in insulin-induced hypoglycemic dogs (hypoglycemia and hyperinsulinemia), and decreased in glucose-insulin infused dogs (hyperglycemia and hyperinsulinemia). These results indicated that brain GI responds inversely to plasma glucose levels in the presence of hyperinsulinemia. Our previous observation suggested that the brain GI was increased in both hyperglycemia and hypoglycemia when insulin was absent. It is deduced that the brain GI increases in either hypoglycemia or hypoinsulinemia which may induce intracellular glycopenia.

We have previously presented (10) evidence that intracerebroventricular administration of porcine glucagon in the dog induces hyperglycemia through the cholinergic and α -adrenergic nervous pathway and, in part, through the adrenal medulla. Although the occurrence of glucagon-like materials in the brain has been confirmed by several investigators including us (1, 4, 6, 9, 13-15), their physiological role in the brain has not yet been determined. Our previous report showed that glucagon immunoreactivity (GI) measured by radioimmunoassay with a C-terminal specific antiserum was increased in the thalamus-hypothalamus in starved and alloxan-treated diabetic dogs (16). These findings strongly suggest that the increment of GI in the thalamus-hypothalamus results from either hypoglycemia (in starved dogs) or hypoinsulinemia (in alloxan-treated diabetic dogs). Since both conditions decreased intracellular glucose utilization, the brain GI may respond to intracellular glucose utilization in the brain.

To confirm this assumption more directly, changes in glucagon-like materials in the brain were investigated during insulin-induced hypoglycemia and glucose-insulin infusion.

MATERIALS AND METHODS

Insulin Induced Hypoglycemia

Four mongrel dogs were intravenously given 20 units of Actrapid Insulin (Novo) after overnight fasting. The venous blood was sampled at 0, 10, 20, and 30 min after injection. Thirty minutes after insulin injection, the dogs were killed by complete blood-letting, and the brain was removed and dissected into the following portions; thalamus-hypothalamus, midbrain, pons and medulla oblongata. These portions of the brain were stored at -20°C until use.

Glucose-Insulin Infusion

Glucose and insulin were infused into another 4

mongrel dogs after overnight fasting. After the administration of glucose (20% solution) at 1.25 g/kg body weight and Actrapid Insulin at 0.1 U/kg body weight, a concomitant infusion of glucose (62.5 mg/kg/min) and Actrapid Insulin (0.417 U/kg/min) was carried out for 30 min. In order to avoid hypokalemia, 20 mEq of potassium chloride were infused for 30 min. Blood samples were collected 10 and 5 min before, and 0, 2, 5, 10, 15, 20, 25, and 30 min after glucose-insulin administration. At the end of the glucose-insulin infusion, the brain was removed and dissected as above. Four other dogs fasted overnight were killed, and the brain was used as controls.

Glucose in the venous blood was determined by the glucose oxidase method. Glucagon immunoreactivity (GI) was determined by radioimmunoassay with C-terminal specific antiserum 30 K (5), and glucagon-like immunoreactivity (GLI) by radioimmunoassay with central portion specific antiserum K4023 (7). Immuno-reactive insulin (IRI) was determined by conventional methods of radioimmunoassay. Each portion of the brain was extracted by Kenny's method (8) with acid ethanol. Statistical significance was examined by Student's *t* test.

RESULTS

Insulin-Induced Hypoglycemia

The plasma level of glucose was decreased from 60 ± 7 mg/dl (mean $\pm$ SD) to 24 ± 5 mg/dl 30 min after the insulin injection (Fig. 1). The plasma level of GI was increased from 117 ± 4 pg/ml to 181 ± 38 pg/ml 30 min after injection (an increase by 54.7%). However, plasma level of GLI did not change during these 30 min.

The GI value in the thalamus-hypothalamus after insulin injection was significantly increased from $2,437 \pm 780$ pg/g to $4,533 \pm 1,677$ pg/g (mean $\pm$ SD) (an increase by 81.4%; Fig. 2). The GI value in the medulla oblongata was also increased. GI in the midbrain and pons did not increase significantly from the control. GLI in all these brain regions did not change after insulin injection (Fig. 3).

Glucose-Insulin Infusion

The plasma level of glucose and IRI was markedly increased under these experimental conditions (Fig. 4). Plasma GI was gradually decreased from 73 ± 37 pg/ml to 42 ± 10 pg/ml 30 min after starting the infusion (a decrease to

Fig. 1 Effect of insulin administration on the plasma level of glucose, glucagon immunoreactivity (GI) and glucagon-like immunoreactivity (GLI). Four dogs fasted overnight were intravenously given 20 units of Actrapid Insulin (Novo). The plasma level of GI, GLI and glucose (PG) was determined at time points indicated. Means $\pm$ SE are given.

57.5%). Plasma GLI was also decreased, but not significantly.

GI in the thalamus-hypothalamus after glucose-insulin infusion was significantly decreased to 720 ± 318 pg/g (a decrease to 29.5%; Fig. 2). GI in the midbrain was also decreased. However, GI in the pons and medulla oblongata did not change significantly. On the other hand, GLI in the thalamus-hypothalamus and midbrain was decreased (Fig. 3), but not in the pons and medulla oblongata.

DISCUSSION

The present experiments show that the concentration of GI in the thalamus-hypothalamus and in the plasma is increased during insulin-induced hypoglycemia, but decreased during glucose-insulin infusion. The possibility that the change in GI in the brain is due to specific or non-specific binding, or contamination of plasma GI to brain tissue seems unlikely for the following

Fig. 2 Effect of insulin administration and glucose-insulin infusion on glucagon immunoreactivity (GI) in the canine brain. For experimental details, see the text. gww, gram wet weight

Fig. 3 Effect of insulin administration and glucose-insulin infusion on glucagon-like immunoreactivity (GLI) in the canine brain

reasons: 1) the concentration of glucagon-like materials in the thalamus-hypothalamus was 30 to 50 times higher than in the plasma, 2) the increase or decrease of GI in the thalamus-hypothalamus was more marked than in the plasma, 3) a specific binding site for glucagon has not been found in the brain, and 4) distribution of glucagon-like substances in the brain,

and changes in the GI concentration in brain regions examined were uneven. The present results indicate that brain GI responds inversely to the plasma glucose level in the presence of hyperinsulinemia. We previously found that the content of GI in the thalamus-hypothalamus was increased in starved and alloxan-treated diabetic dogs (16). The starvation induces

Fig. 4 Effect of glucose-insulin infusion on the plasma level of glucose (PG), insulin (IRI), glucagon immunoreactivity (GI) and glucagon-like immunoreactivity (GLI). Four dogs fasted overnight were given an intravenous injection of glucose (1.25 g/kg body weight) and Actrapid Insulin (Novo; 0.1 U/kg body weight), and then an infusion of glucose (62.5 mg/kg/min) and Actrapid Insulin (0.417 U/kg/min) for 30 min.

hypoglycemia and hypoinsulinemia; the alloxan treatment may induce hyperglycemia and hypoinsulinemia, and the massive injection of insulin induces hypoglycemia and hyperinsulinemia. Under these three conditions, there is both hypoglycemia and hypoinsulinemia together, or either hypoglycemia or hypoinsulinemia alone. Several workers have reported that insulin can affect glucose deposition in the brain (2, 3, 12). If insulin plays a role in glucose transport from the plasma to brain tissues, the decrease in either glucose or insulin may cause intracellular glucose deprivation. In contrast, glucose-insulin infusion resulted in hyperglycemia and hyperinsulinemia. In this case, the intracellular glucose level may increase if insulin serves in glucose transport from the plasma to the brain tissue. Changes in GI concentration in the thalamus-hypothalamus during insulin-induced hypoglycemia and glucose-insulin infusion may thus reflect the intracellular glucose level. We therefore deduce that brain GI increases in either hypoglycemia or

hypoinsulinemia, which may induce intracellular glycopenia.

Although GLI in the brain did not change during insulin-induced hypoglycemia, GI in the thalamus-hypothalamus was significantly increased, which resulted in a decrease of the GLI/GI ratio. We also found that the GLI/GI ratio in the thalamus-hypothalamus was decreased after starvation or alloxan treatment (16). These observations suggest that the posttranslational processing of glucagon is enhanced during insulin-induced hypoglycemia as well as during starvation and alloxan treatment.

The content of GI in the medulla oblongata was also increased. Although evidence concerning the role of the medulla oblongata, especially the nucleus tractus solitarius, in glucose metabolism has recently been reported (11), significance of GI in the medulla oblongata remains to be elucidated.

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