

The Dual Mechanism of Glucagon-Induced Hyperglycemia

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Summary. Recently glucagon has been found in several extrapancreatic tissues, mainly in the brain and gut. This wide distribution of glucagon raises the possibility that glucagon may exert its action in several ways at different places. As for glucose production in the liver, glucagon secreted from the pancreas acts directly on the liver either through cyclic AMP-mediated glycogenolysis or gluconeogenesis. Intravenous (iv) administration of glucagon produced transient hyperglycemia; glucagon-induced hyperglycemia occurs at first and is immediately suppressed by glucagon-induced hyperinsulinemia. In the brain, glucagon is localized mainly in the hypothalamus, thalamus and brain stem—the center of the autonomic nervous system—where glucagon is modulated by the changes in glucose homeostasis, starvation or glucose-insulin administration. The intracerebroventricular (icv) administration of glucagon in the rat induces more prolonged hyperglycemia than that induced by iv injected glucagon. This hyperglycemia is not associated with hyperglucagonemia or hyperinsulinemia, suggesting that icv administered glucagon does not leak into the peripheral circulation. Furthermore, icv glucagon-induced hyperglycemia is inhibited completely by pretreatment with atropine, phentolamine or hexamethonium, partially by bilateral adrenalectomy, but not by propranolol. Thus, glucagon-induced hyperglycemia seems to operate by a dual mechanism: a direct effect on the liver, and an indirect effect through cholinergic and alpha-adrenergic neural pathways to the liver.

Introduction

Glucagon was first found as a hyperglycemic factor in a side-product of insulin from the porcine pancreas. The main source of glucagon together with insulin is the pancreas, which is located upstream of the liver. Therefore, these two peptides are directly sent to the liver and exert their antagonistic actions in glucose metabolism in the liver.

The glycogenolytic action of glucagon in the liver has been determined to act through the chain reactions of receptor binding, the activation of adenylate cyclase, of protein kinase and of phosphorylase.^{1,2)} The activation of gluconeogenesis by glucagon has also been established³⁾; thus, the main action of glucagon appears to be confirmed.²⁾

Recently, an increasing number of peptides have been found both in the gut and brain,⁴⁻⁶⁾ and referred to as brain-gut peptides. Some of these induce hyperglycemia when administered either centrally or peripherally.^{7,8)} Glucagon-like substances are also found immunochemically and immunohistochemically in the brain of mammals,⁹⁻²¹⁾ avians²²⁾ and invertebrates, including insects.^{23,24)} It is therefore possible that glucagon in the brain may act on the glucose metabolism through the central nervous system.

In this study, we aim to clarify the two different mechanisms of glucagon-induced hyperglycemia, by reviewing published reports and referring to our own data.

Modulation of plasma glucose by intravenous administration of glucagon

Human study

When 1 mg glucagon was administered intravenously, plasma glucose increased immediately and peaked at 15 min, followed by a rapid reduction to the basal level within 60 min.²⁶⁾ A great increase in plasma glucose levels associated with an increase in insulin was also noted.²⁷⁾ The rapid decline of plasma glucose levels after transient hyperglycemia induced by intravenous (iv) glucagon could be explained by glucagon-induced (reactive) hyperinsulinemia. Parallel increases in plasma cyclic AMP and glucose suggest a close relationship between cyclic AMP and glucose production.²⁶⁾

Rat experiment

An intravenous injection of 10 ng glucagon into the rat produced a minimal increase in plasma glucose at 5 to 10 min, followed by a rapid decline to lower than the basal level at 30 to 120 min.²⁸⁾ The iv injection of 100 ng glucagon induced a sharp rise in plasma glucose at 5 to 10 min, followed again by a rapid decline to below the basal level at 30 to 120 min²⁹⁾ (Fig. 1).

Plasma immunoreactive glucagon (IRG) levels increased from the basal level of 105 ± 77 pg/ml to a peak of 971.5 ± 87.0 pg/ml at 5 min, and returned to the basal level at 30 min. Plasma immunoreactive insulin (IRI) levels were initially 68.4 ± 32.4 μ U/ml, increasing to 288.9 ± 92.5 μ U/ml at 5 min, and 158.4 ± 98.4 μ U/ml, still higher than the basal level, at 30 min (Fig. 2).

These results again suggest that the rapid decline of plasma glucose is due to hyperinsulinemia responsive to exogenous glucagon.

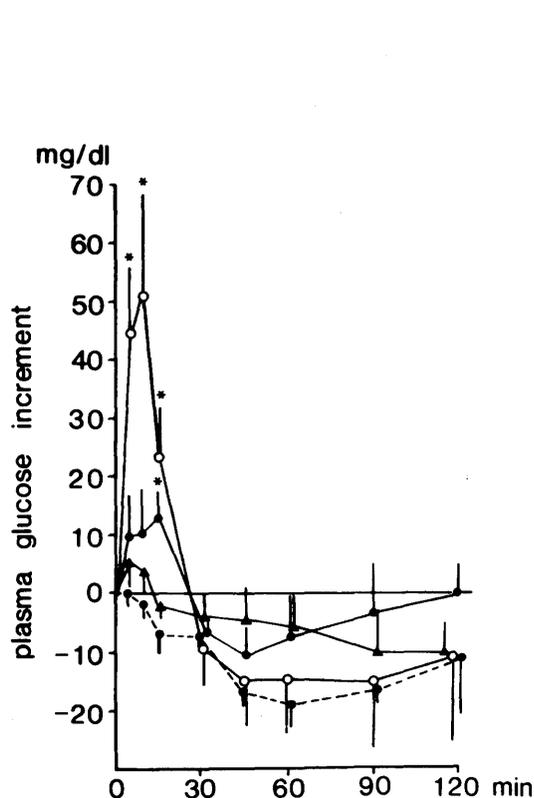


Fig. 1. Modulation of plasma glucose levels by the intravenous injection of saline (dotted line), 10 ng (●—●) and 100 ng (○—○) glucagon, and intramuscular injection of 100 ng glucagon (▲—▲) into the rat. * represents $p < 0.05$.

Glucose production from the liver by peripheral administration of glucagon

The basal glucose output from the isolated perfused rat liver was stable at 0.39 ± 0.04 μ mole/g liver/min.²⁵⁾ When 10^{-11} M glucagon was infused, glucose output increased gradually from 2 min and reached a maximum at 10 min, as depicted in Fig. 3. A significant increment of glucose output was obtained with 10^{-11} M glucagon, and reached the maximal level of 2.56 ± 0.14 μ mole/g liver/min with 10^{-10} M. A higher concentration of glucagon produced a more prolonged response. The total amount of glucose output increased in a dose dependent manner from 10^{-11} M to 10^{-8} M of glucagon.²⁵⁾

On the other hand, the cyclic AMP level in the effluent was below the detection limit in the absence of glucagon, but increased significantly with 10^{-10} M or higher concentrations of glucagon.²¹⁾ However, the cyclic AMP output increased immediately after

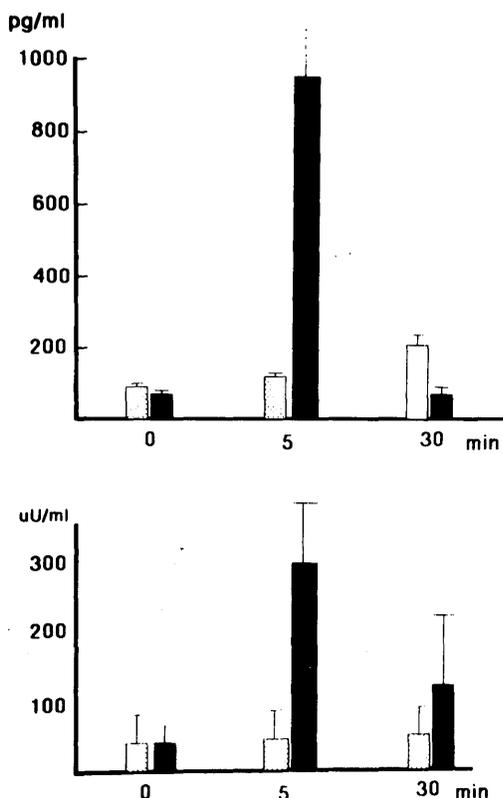


Fig. 2. Modulation of plasma levels of immunoreactive glucagon (IRG) (upper panel) and immunoreactive insulin (IRI) (lower panel) by the intravenous (solid column) or intracerebroventricular (shaded column) administration of 100 ng glucagon. Each value represents the mean \pm SD.

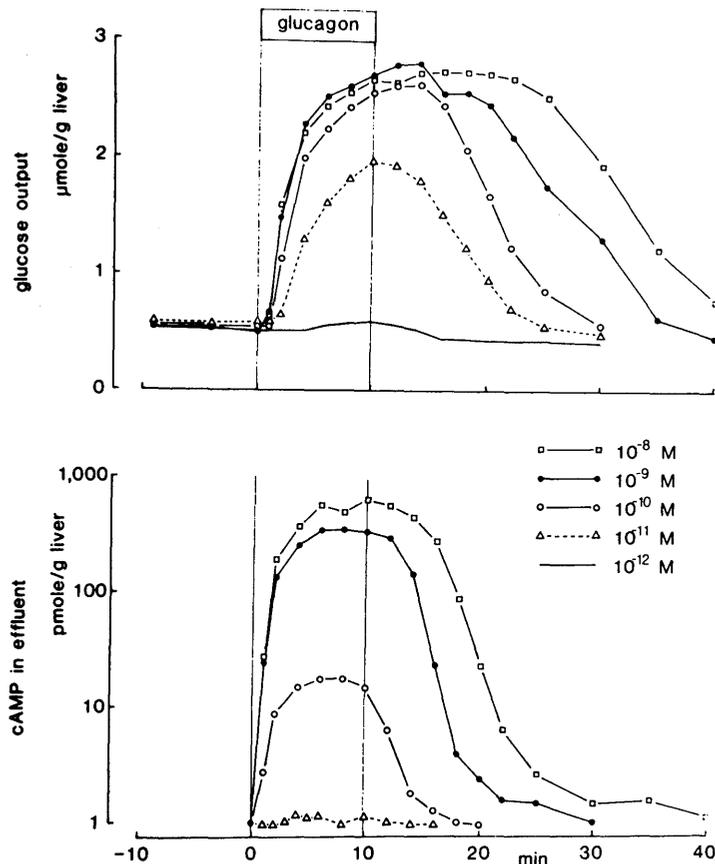


Fig. 3. Glucagon-induced glucose and cyclic-AMP output from the isolated perfused rat liver. Each point represents the mean of 3-4 experiments.

glucagon infusion and peaked at 3 to 5 min. Although a minimal concentration of glucagon required to increase cyclic AMP output was 10 times more than that required to increase glucose output, the content of cyclic AMP in the liver increased significantly with 10^{-11} M glucagon.²⁵⁾

These sequential changes in cyclic AMP- and glucose-output suggest that glucagon-induced glucose output from the liver is mediated by cyclic AMP. Considering the location of the pancreas, pancreatic glucagon would appear responsible for this mechanism.

Glucagon in the brain

As previously reported, glucagon-like substances have been found in the brains of various mammals.⁹⁻²¹⁾ Fig. 4 represents the contents of IRG and glucagon-like immunoreactivity (GLI) in the brain of the dog,

pig, cattle, rat and man.^{10,12)} Of the animals examined, the highest concentrations of both IRG and GLI were found in the dog. In all animals examined, the highest concentrations of both peptides also were found in the thalamus-hypothalamus, followed by the brain stem. Significant amounts of IRG and GLI were also detected in the canine, porcine and bovine spinal cords. However, no immunoreactant was found in the pituitary gland, basal ganglia or the cerebral cortex in any animal examined except for the rat.

The localization of glucagon-like substances was found immunohistochemically in the perikarya and nerve viscosities,^{11,13-19,32)} and immunochemically in the synaptosome³¹⁾ in the brains of several mammals, especially in the hypothalamus and brain stem. More recently, the production of proglucagon-derived peptides has been confirmed by the molecular biology technique.^{20,21)}

Moreover, the contents of glucagon-like substances

Table 1. Effects of starvation, alloxan treatment, insulin-induced hypoglycemia or insulin-glucose infusion on contents of IRG and GLI in the canine hypothalamus.

	IRG	GLI
Control	1690 ± 291	10730 ± 683
Starvation for 3 days	1632 ± 870	6704 ± 2875
Starvation for 6 days	2770* ± 666	8415 ± 917
Alloxan treated	3162* ± 164	14110 ± 3082
Insulin infusion	4533* ± 1677	14300 ± 2880
Insulin-glucose infusion	720* ± 31	2830* ± 740

Values are shown as the picogram equivalent of glucagon per gram of wet weight (mean ±SD) * indicates $p < 0.05$ versus control.

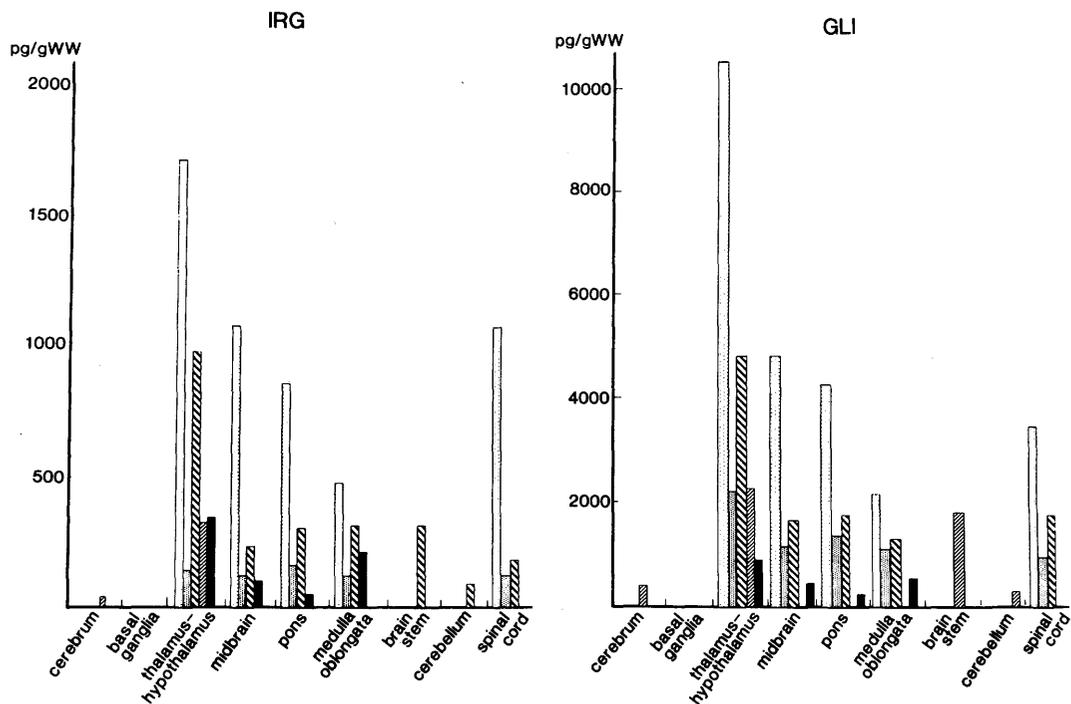


Fig. 4. The contents of immunoreactive glucagon (IRG) (right panel) and glucagon-like immunoreactivity (GLI) (left panel) in the brain of the dog (▨), pig (▩), cattle (▧), rat (▦) and man (■).

—IRG and GLI—in the canine thalamus-hypothalamus changed under several experimental conditions as shown in Table 1.

The IRG content was not increased after starvation for 3 days, but increased by $84.0 \pm 34.4\%$ after starvation for 6 days²⁹⁾ (hypoglycemia + hypoinsulinemia). It was also increased by 87.1% in alloxan diabetes²⁹⁾ (hyperglycemia + hypoinsulinemia) and by 81.4% by insulin-induced hypoglycemia³⁰⁾ (hypoglycemia + hyper-

insulinemia), but decreased to $29.5 \pm 6.5\%$ by the combined infusion of insulin and glucose (hyperglycemia + hyperinsulinemia).³⁰⁾ The GLI values were not changed after starvation for both 3 and 6 days, in alloxan diabetes or by insulin administration, but decreased significantly by glucose-insulin infusion.

Glucagon-like substances appeared to respond to changes in the glucose metabolism, since these increased in both hyperglycemia and hypoglycemia in

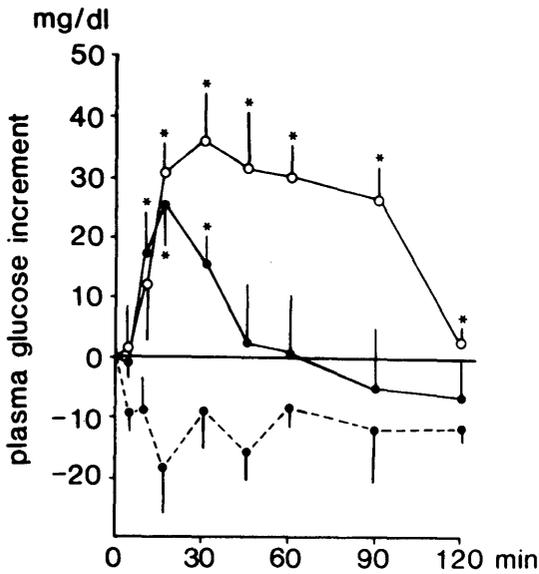


Fig. 5. Modulation of plasma glucose levels by the intracerebroventricular administration of saline (dotted line), and 10 ng (●—●) and 100 ng (○—○) glucagon. Each point represents the mean of 4 experiments ± SD.

the absence of insulin, and changed conversely to plasma glucose levels with an excess of insulin. These changes presumably reflect intracellular glucose utilization.

Hyperglycemic effects of glucagon administered centrally

With the intracerebroventricular (icv) injection of saline into the rat (the control), plasma glucose levels decreased gradually over 120 min, as shown in Fig. 5. Although the intravenous injection of glucagon induced a transient sharp rise in plasma glucose as shown in Fig. 2, the icv administration of 10 ng glucagon did not induce a significant elevation of plasma glucose for the initial 5 min, but increased at 10 to 60 min. The icv administration of 100 ng glucagon induced a more prolonged hyperglycemia at 10 to 90 min, compared to the icv 10 ng glucagon and the iv 100 ng glucagon²⁸⁾ (Fig. 1).

Maximal increments were 25.7 ± 7.2 mg for 10 ng glucagon and 35.6 ± 8.0 mg/dl for 100 ng glucagon at 30 min (Fig. 1).

The plasma IRI and IRG levels were unchanged following the icv administration of 100 ng glucagon, contrasting with their marked increase following the iv injection of 100 ng glucagon,²⁸⁾ as shown in Fig. 2.

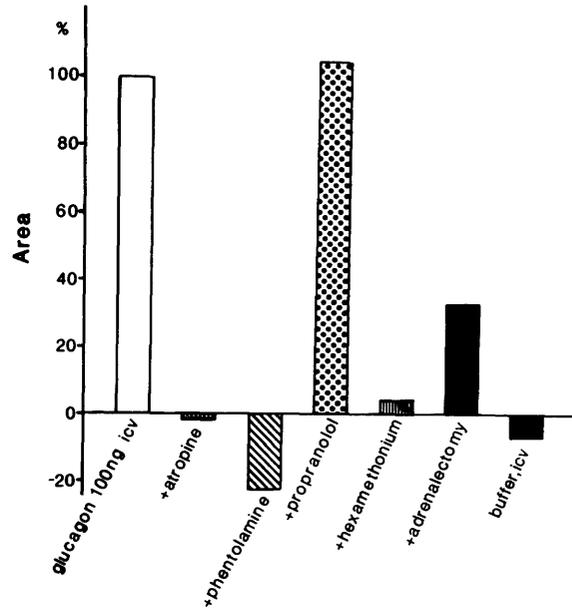


Fig. 6. Effect of autonomic nerve blockades and adrenalectomy on hyperglycemia induced by the intracerebroventricular administration of 100 ng glucagon.

Thus, icv glucagon-induced hyperglycemia was not due to a leakage of glucagon into the peripheral circulation.

Fig. 6 represents the percentages of changes by various medications in the plasma glucose area above the basal level.²⁸⁾ The hyperglycemic effect from the icv injection of 100 ng glucagon was eliminated completely by pretreatment with atropine, phentolamine or hexamethonium, and suppressed to $28.1 \pm 10.1\%$ by bilateral adrenalectomy, but not by propranolol ($108 \pm 21.4\%$). Thus, the glycemic effect of icv glucagon was mediated via alpha-adrenergic and cholinergic nervous pathways and partly through the adrenal medulla. In addition, an inhibition in insulin secretion, possibly due to alpha-adrenergic action, may be at least partially involved in producing hyperglycemia, because plasma levels of IRI did not increase despite a hyperglycemic state during the entire period.

Conclusion

Glucagon was found to induce hyperglycemia by a dual mechanism. Glucagon secreted from the pancreas reached the liver through the portal vein and induced transient hyperglycemia. A rapid decline in glucose may be due partly to the reactive hyperin-

sulinemia and partly to the rapid degradation of glucagon in the liver. Glucagon produced in the brain acts on the liver through the nervous pathway(s) and induces a long-lasting hyperglycemia in which the adrenal medulla may partially participate. Glucagon in various organs could contribute to glucose homeostasis. As discussed in this paper, transient hyperglycemia may be induced by pancreatic glucagon, and persistent hyperglycemia by brain glucagon.

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