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Stimulation of Gastric Glucagon Secretion by Epinephrine Administration in Dogs

E. Blázquez* and L. Muñoz-Barragán**

Departamento de Bioquímica
Facultad de Medicina
Universidad Complutense
28040 Madrid (Spain)

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To determine the response of gastric A-cells to adrenergic substances, immunoreactive glucagon was determined simultaneously in the jugular vein and in the left gastroepiploic vein of totally depancreatized dogs. Under basal conditions a significant gradient of glucagon concentrations between the jugular and gastric veins was observed, whereas plasma insulin values were almost undetectable. Intravenous administration of epinephrine elicits a prompt and significant increase in glucagon concentrations in the gastric vein which persist during the time of hormone infusion. To ensure adequate adrenergic blockade, blockers were infused before epinephrine administration. Accordingly, after phentolamine, the infusion of epinephrine failed to increase gastric glucagon concentrations, while after propranolol, epinephrine induced a significant release of gastric glucagon. These results indicate that epinephrine stimulates gastric glucagon secretion and that this effect is mediated through α-adrenergic receptors.

Key words: Gastric glucagon, Secretion, Epinephrine, Adrenergic blockers.

There have been several reports on the existence of A cells in the stomach of both human and animal species used in the laboratory (1, 12, 17). These cells produce a polypeptide with the same physicochemical, immunological and biological properties as pancreatic glucagon (21), which is released to the bloodstream in response to specific stimuli (16). Accordingly gastric glucagon secretion is modified by the effects of nutrients and several regulatory gut peptides (18). Extrapancreatic glucagon also contributes significantly to the hyperglucagonemia of insulin-deprived alloxan-diabetic dogs (3) and represents the major source of the circulating hormone in totally depancreatized animals of this species.

Although there is abundant information concerning adrenergic influence on pancreatic A and B cells, only a few reports are related with the role of norepinephrine on the release of gastric glucagon (13, 14). Even so, these studies were carried out in normal dogs, in which gastric A-cells contribute minimally to the circulating

* To whom all correspondence should be addressed.

** Present address: Departamento de Anatomía e Histología humana, Facultad de Medicina, 37007 Salamanca (Spain).

glucagon levels and its response to different stimuli is very poor (15, 18). The present studies were therefore designed to examine the effects of epinephrine with or whithout pretreatment by α and β blockers on gastric glucagon secretion.

Materials and Methods

Animals. — Mongrel dogs weighing 18 to 24 kg were used. The animals were fed a diet of 1 can of Purine Dog Chow plus 1 can of meat at 7 a.m. and 3 p.m. Diabetes was induced following total pancreatectomy, and thereafter the dogs received 3-5 U of NPH insulin twice daily for 10-14 days. Without insulin treatment fasting plasma glucose levels ranged from 250 to 500 mg/dl.

With the totally depancreatized dogs under nembutal anaesthesia a Teflon catheter was passed through the jugular vein and anchored in place and a silastic catheter (0.04 × 0.085 inches, Dow Corning, Midland, Michigan) was implanted in the left gastroepiploic vein draining the gastric fundus and thus permitting simultaneous sampling of blood from the effluent of the gastric fundus and the systemic circulation. Regular insulin which had substituted NPH insulin 48 hours before implantation of the indwelling catheters was administered at a dose of 3 U after meals. Insulin treatment was discontinued 18 h before each experiment.

All experiments were performed in conscious dogs. To avoid anemia, the dogs received their own red blood cells suspended in a sterile saline (0.9 %) solution after each experiment. Any animals with haematocrit values below 30 %, leukocyte count of about 20,000 or showing clinically evident signs of illness were excluded from the experimental groups.

Analytical determinations. — Plasma glucose was determined by the glucose oxidase method, using a glucose analyzer (Beckman Instruments Inc., Fulleston, Ca.). Insulin was determined by the radioimmunoassay of HERBERT *et al.* (10). Glucagon immunoreactivity was measured according to FALOONA and UNGER (5), using 30 K rabbit antiserum which is considered to be reactive with the C-terminal portion of the glucagon molecule and crossreacts only very weakly with glucagon-like immunoreactivity (GLI).

Results are expressed as the mean ± SEM. Statistical comparisons between groups were performed using Student's t test.

Results

Effect of intravenous epinephrine infusion on plasma glucose, insulin and glucagon levels of depancreatized dogs.— To determine the effect of intravenous epinephrine infusion on gastric glucagon release, blood specimens were collected simultaneously at 10 minutes intervals from a gastric vein and from the jugular vein. As is shown in fig. 1, there was a gradient of glucagon concentrations between the gastric and jugular veins, both in basal and epinephrine-stimulated situations. A rise in plasma glucagon in the gastroepiploic vein during intravenous epinephrine infusion occurred despite coexistent hyperglycemia and also in the presence of minimal amounts of circulating insulin.

Effect of intravenous epinephrine infusion after the treatment with alpha or beta blockers, on plasma glucose, insulin and glucagon levels of depancreatized dogs.— To determine if the effect of epinephrine on gastric glucagon release was achieved through an alpha or beta receptor or both, we infused this hormone with phentolamine or propranolol (figs. 2 and 3). Blockers administration was carried out before, during and after the infusion of epinephrine. After phentolamine infusion,

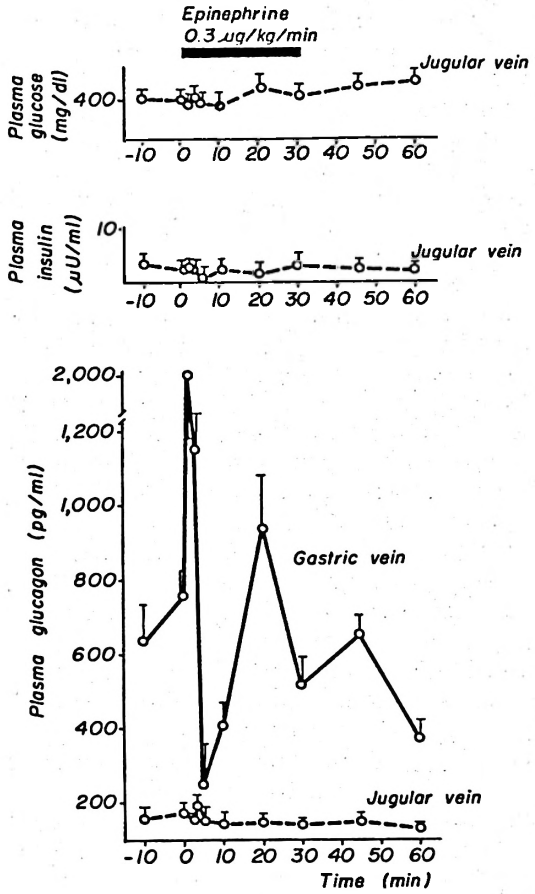

Fig. 1. *Effect of intravenous epinephrine infusion (0.3 µg/kg b.w./min) for 30 min on circulating glucose, insulin and glucagon levels of insulin-deprived depancreatized dogs.*
(Means ± SEM, n = 3.)

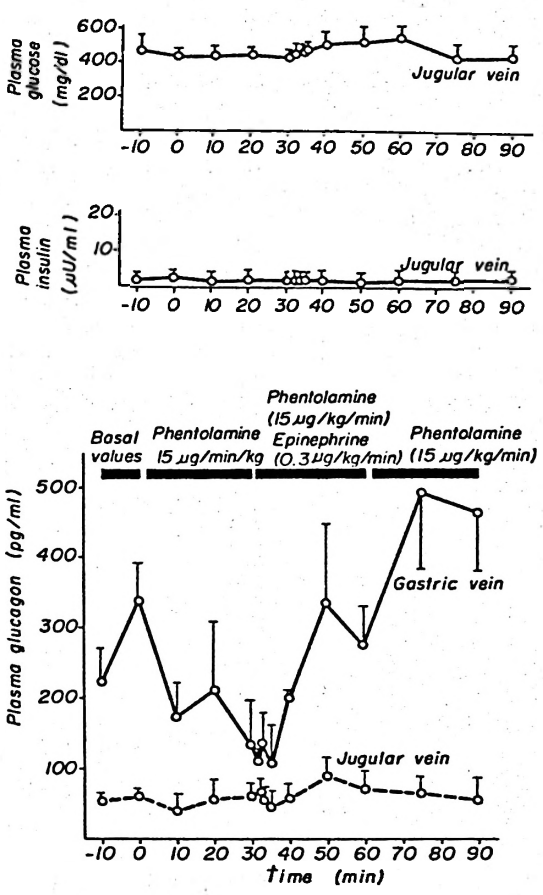

Fig. 2. *Effect of intravenous epinephrine infusion (0.3 µg/kg b.w./min) for 30 min, after α-adrenergic blockade with intravenous phentolamine administration (15 µg/kg b.w./min) for 30 min on circulating glucose, insulin and glucagon levels of insulin-deprived depancreatized dogs.*
(Means ± SEM, n = 3.)

epinephrine failed to increase gastric glucagon release (fig. 2), although after epinephrine infusion a small rebound effect was observed. However, the administration of propranolol cannot avoid the significant increase of gastric glucagon secretion induced by epinephrine (fig. 3).

Discussion

The experimental model used in this study has been previously validated by us in order to determine *in vivo* the release of gastric glucagon in response to specific stimuli (2, 3, 16, 18). In particular the experiments were conducted in conscious dogs, thus avoiding the effect of anaesthetic on the release of glucagon (4). Moreover the totally depancreatized dogs were deprived of regular insulin administration for at least 18 hours before the experiments in order to avoid interferences

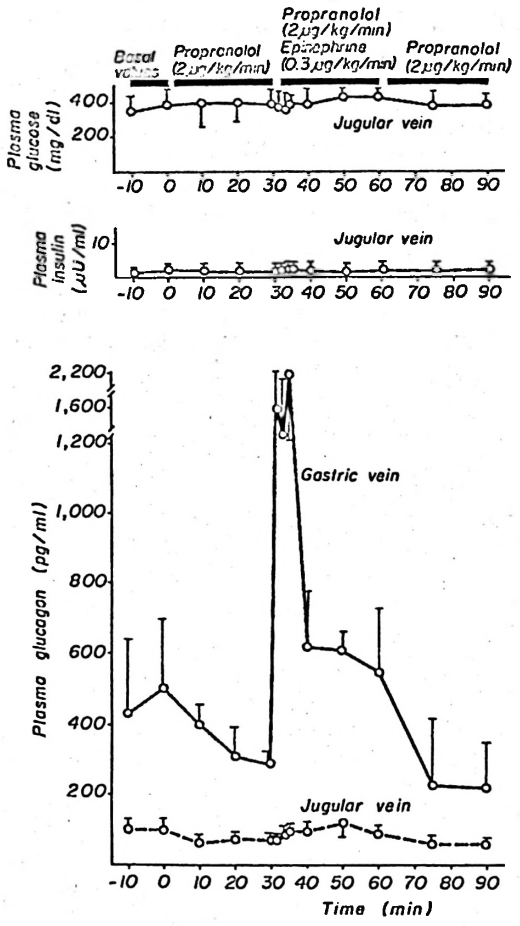

Fig. 3. *Effect of intravenous epinephrine infusion (0.3 µg/kg b.w./min) for 30 min, after β-adrenergic blockade with intravenous propranolol administration (2 µg/kg b.w./min) for 30 min on circulating glucose, insulin and glucagon levels of insulin-deprived depancreatized dogs.*
(Means ± SEM, n = 3.)

in the release of gastric glucagon. Also, in the digestive tract of the dog A-cells are mainly located in the stomach (1), while cells containing GLI rather than glucagon have been detected in the small intestine (19). As it has been described before in depancreatized dogs (25), the changes of glucagon concentrations in jugular and gastric veins in response to several stimuli are related to modifications of true glucagon.

In our experiments, there was a gradient of glucagon concentrations between the gastric and jugular veins, both in basal and glucagon-stimulated situations. Epinephrine at a dose that approximates the physiologic response to stress (23) increased gastric glucagon release. A rise in plasma glucagon in the gastroepiploic vein during intravenous epinephrine infusion occurred despite coexistent hyperglycemia and also in the presence of minimal amounts of circulating insulin. Changes in plasma glucagon concentrations cannot be accounted for by the effect of epinephrine on blood flow, since several authors have reported that neither this substance nor adrenergic blockers at the concentrations used here modify gastric blood flow (22, 27). As it happens with gastric glucagon, epinephrine also increases the secretion of pancreatic glucagon in man and experimental animals while it inhibits insulin release (7, 20). Since epinephrine is a mixed adrenergic agent, increased glucagon release may have been mediated through an alpha or beta receptor or both. To determine if the effect of epinephrine on gastric glucagon release was achieved by either one this hormone was infused with phentolamine or propranolol. To ensure adequate blockade, blocker administration was started before the infusion of epinephrine. It is moreover known that the doses of alpha and beta blockers used do not modify blood flow through the stomach (26, 27). After phentolamine infusion, epinephrine failed to increase gastric glucagon release although after epinephrine infusion a small rebound effect was observed. However, after propranolol administration, epinephrine induced a significant increase in gastric glucagon secretion. On comparing these results with those obtained for pancreatic glucagon, several authors have reported that the effects of an α-adrenergic agonism on pancreatic A-cell secretion are stimulatory in

man (6, 20, 24) ducks, rats and dogs. On the other hand, it has also been suggested that β-adrenergic agonism either inhibits glucagon secretion in man and ducks (6, 24), or stimulates glucagon in man (8), rats (15) and dogs (11). By contrast, our results suggest that this effect may be mediated through α-adrenergic receptors. Our results contrast with those of LEFÈBVRE and LUYCK (14), who have reported that the canine gastric A-cell is not influenced by endogenous or exogenous norepinephrine in the isolated, perfused stomach of normal dogs. This apparent paradox could be related to the fact that gastric A-cells from normal dogs have a poor response to specific stimuli (16, 18) as compared with diabetic animals (2, 3, 18).

Resumen

Se determina la respuesta de las células A gástricas a sustancias adrenérgicas, valorando simultáneamente el glucagón inmunorreactivo en la vena yugular y en la gastroepiploica izquierda de perros totalmente pancreatectomizados. En condiciones basales, se observa un significativo gradiente de las concentraciones de glucagón entre las venas gástrica y yugular, mientras que los valores de insulina son casi indetectables. La administración intravenosa de adrenalina produce una rápida y significativa elevación de las concentraciones de glucagón en la vena gástrica que persiste durante la infusión. Para asegurar un bloqueo adrenérgico adecuado, los agentes bloqueantes se infunden antes de la administración de adrenalina. De esta forma después de la administración de fentolamina, la infusión de adrenalina no aumenta las concentraciones de glucagón gástrico, mientras que después de la administración de propranolol, la adrenalina induce una significativa liberación de glucagón gástrico. Los resultados indican que la adrenalina estimula la secreción de glucagón gástrico y que este efecto es mediado a través de receptores alfa-adrenérgicos.

Palabras clave: Glucagón gástrico, Secreción, Adrenalina, Bloqueantes adrenérgicos.

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