Arguments for a Regulation of Pancreatic Glucagon Secretion by Circulating Plasma Free Fatty Acids (34511)

A. S. LUYCKX[1] AND P. J. LEFEBVRE
(Introduced by A. E. Renold)

Secteur Diabétologie, Institut de Médecine, Département de Clinique et de Pathologie Médicales, Université de Liège, Belgium

The availability of a sensitive radioimmunoassay for glucagon (1) has permitted the demonstration that pancreatic glucagon secretion is stimulated by hypoglycemia (2–4), infusions or ingestion of amino acids (5, 6), and injection of pancreozymin (7), and reduced by an elevation of blood sugar (2–4). In addition, Seyffert and Madison (8) and Madison *et al.* (9) have recently demonstrated that an increase in plasma free fatty acids (FFA) obtained by simultaneous infusion of triglycerides and heparin, induces, in the dog, a fall in peripheral plasma glucagon levels. Glucagon is a potent lipolytic hormone (10–12) both *in vivo* and *in vitro*. In the past few years, we have advanced several arguments in favor of a physiologic role of glucagon in lipid mobilization from adipose tissue (11–13). The suppression of pancreatic glucagon secretion by a high FFA level is further evidence for such a role. In order to understand more completely the interrelationship between glucagon and FFA, we have investigated the effect of lowering plasma FFA on pancreatic glucagon secretion.

Materials and Methods. Mongrel dogs weighing about 20 kg (15.5-23.5 kg) and fasted for 18 hr were used for these experiments. Catheters were inserted into the femoral artery and vein and into a jugular vein during anesthesia induced by intravenous injection of 30 mg/kg body weight of pentobarbital. At laparotomy, a polyethylene catheter (Clay Adams PE 200) was introduced through its main duodenal branch into the superior pancreaticoduodenal vein in the same direction as the blood flow. In some experiments, the catheter was introduced into the proximal part of the superior pancreaticoduodenal vein (PDV) about 2 cm from the portal vein. In these experiments, the PDV was ligated, the pancreatic venous effluent drained off and reinfused through the splenic vein as previously described (14). This permits measurement of pancreatic blood flow. Blood samples were drawn simultaneously from the femoral artery (9 ml) and, by free flow, from the PDV (4 ml) into tubes containing 0.2 ml of Trasylol (5000 U/ml) and 0.02 ml of heparin (5000 U/ml). In dogs in which the pancreaticoduodenal effluent was diverted, coagulation was prevented by injection of heparin (500 U/kg) immediately after cannulation of the vessel. In the other type of experiments, the catheter was kept patent between blood samples by a slow infusion of saline. Thirty minutes after the end of the surgical manipulation, three blood samples were withdrawn at 7.5-min interval to provide basal values. At zero time, nicotinic acid (1 mg/kg) was given by rapid intravenous injection into the jugular vein and followed by a constant infusion of 0.2 mg/kg body weight/min until the end of the experiment, 90 min later. At 30 min, a triglyceride (TG) emulsion (Lipiphysan Egic) was infused into the jugular vein at a rate of 0.075 ml/kg/min. Large amounts of heparin, 50 U/kg/min, were simultaneously administered. The TG-heparine infusion was maintained for 30 min. Blood samples were drawn from the femoral artery and PDV at 10, 20, 30, 40, 50, 60, 75, and 90 min. Collected blood was immediately replaced by an equivalent amount of blood obtained from a donor. Glucose concentrations

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[1] Chargé de Recherches du Fonds National de la Recherche Scientifique, Belgium.

in arterial plasma were measured by the method of Hoffman (15) adapted to the AutoAnalyzer (Technicon) and FFA by the method of Dole and Meinertz (16). Plasma amino nitrogen was determined according to the method of Malangeau *et al.* (17). Plasma glucagon was assayed by radioimmunoassay using goat antiglucagon serum, ^{131}I-labeled pork glucagon, pork glucagon standards, and descending chromatography on Ecteola paper (18) for separation of free and antibody-bound labeled glucagon. Plasma insulin was assayed by a modification of the double-antibody method of Morgan and Lazarow (19). Both hormones were measured in arterial and PDV plasma. All samples from a single experiment were assayed in the same series using the same standard curve.

Results. The rapid injection, followed by constant infusion of nicotinic acid induced a progressive lowering in plasma FFA concentration which reached its nadir of 32-60% of the basal values after 30 min (Table I). Infusion of TG–heparin promptly reversed this effect and increased mean plasma FFA level to 156 and 172% of mean basal values at 50 and 60 min, respectively. When this infusion was interrupted at min 60 there was a rapid fall in FFA concentrations. In these experiments, PDV blood samples were obtained during free flow of blood, and mean basal value for glucagon in PDV plasma was 0.82 ± 0.11 mμgEq/ml ($n = 15$). Administration of nicotinic acid and the subsequent fall in plasma FFA concentrations was accompanied by a rapid rise of PDV glucagon concentration to 144-206 (mean: 172) % of the basal value at min 20 and to 129–1220 (mean: 366) % of the basal level at min 30 (Fig. 1). When TG–heparin were infused, there was a progressive fall in plasma glucagon in 4 of 5 dogs, and when discontinued, there was a rapid and pronounced rise of plasma glucagon in all of the animals. Arterial plasma glucagon concentrations in our operated dogs ranged between 0 and 0.5 mμgEq/ml. Infusion of nicotinic acid during the first 30 min of the experiments produced only inconstant rise in peripheral plasma glucagon. This was also the case during the 60- to 90-min period. Nevertheless, the highest value for arterial plasma glucagon was reached at 90 min and is statistically different from the zero time value ($p < .05$). A slight and inconstant fall in blood sugar was observed during the first 40 min of the experiment but subsequently returned to basal values. Neither pancreatic nor peripheral plasma insulin levels changed during the course of the experiment. Nevertheless, a moderate but not significant increase in PDV insulin occurred during and after the TG–heparin infusion.

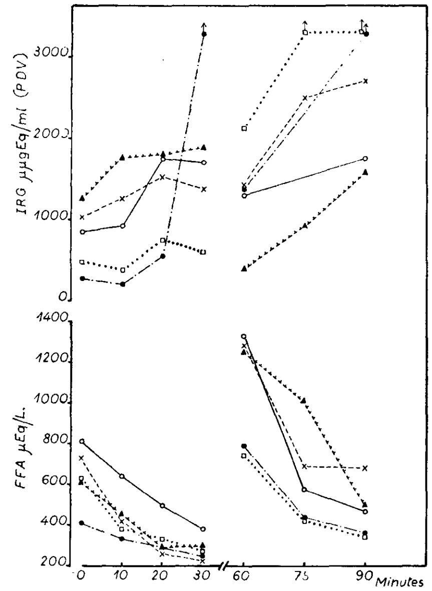

FIG. 1. Changes in PDV plasma glucagon concentrations (IRG) and arterial plasma FFA levels. On the left, the fall in FFA was induced by injection/infusion of nicotinic acid; on the right, the fall in plasma FFA followed cessation of triglyceride emulsion–heparin infusion.

Discussion. Although occurring rapidly, the variations of FFA concentrations observed during the experiments reported are relatively small (235-1335 μeq/liter) and of the same order of magnitude as those measured under physiological conditions. In each dog a fall in FFA plasma levels on two separate occasions was followed by a marked rise in PDV glucagon concentration. On the other hand, during induced elevation of FFA level there was an unequivocal reduction in PDV glucagon in four of the five animals studied. In view of the mirror image be-

TABLE I. Effect of Changes in Plasma FFA Concentration Upon Arterial Plasma Glucose, Aminonitrogen, Insulin (IRI), and Pancreaticoduodenal Vein Plasma Insulin and Glucagon (IRG).

							⟵ TG + Heparin ⟶				
	Basal values			Nicotinic acid infusion (min)							
	–15	–7.5	0	10	20	30	40	50	60	75	90
Arterial glucose mg/100 ml	84.2 ±7.7	84.2 ±8.2	86.4 ±9.7	84.7 ±10.7	82.0 ±9.9	80.1 ±9.6	78.5 ±10.3	81.3 ±12.0	81.7 ±13.0	84.4 ±14.5	87.2 ±14.6
Arterial plasma FFA μEq/liter	708 ±64	640 ±77	614 ±79	438 ±53	374 ±53	276 ±27	819 ±143	998 ±124	1082 ±131	637 ±108	471 ±59
Amino nitrogen mg/liter	38.5 ±3.9	42.0 ±2.6	39.5 ±3.0	37.1 ±1.7	36.7 ±4.6	34.9 ±3.4	45.2 ±2.9	42.0 ±3.4	41.2 ±4.1	39.1 ±3.6	44.0 ±6.3
PDV plasma IRI μU/ml	372.4 ±149.7	214.5 ±59.0	251.2 ±66.0	299.1 ±111.6	263.4 ±102.7	286.1 ±134.5	323.3 ±167.5	358.6 ±133.5	441.8 ±146.8	530.1 ±151.8	647.4 ±281.0
Arterial plasma IRI μU/ml	24.7 ±5.1	24.4 ±3.9	23.4 ±4.7	21.2 ±4.0	17.7 ±2.6	18.7 ±3.7	19.9 ±1.3	23.9 ±5.4	28.5 ±11.0	27.0 ±10.9	31.6 ±14.5
Arterial plasma IRG mμg/ml	0.33 ±0.05	0.33 ±0.05	0.25 ±0.12	0.18 ±0.06	0.25 ±0.12	1.0 ±0.8	0.25 ±0.06	— —	— —	0.25 ±0.10	1.02 ±0.27
PDV plasma IRG mμg/ml	0.90 ±0.20	0.74 ±0.18	0.82 ±0.20	0.94 ±0.25	1.30[a] ±0.23	1.78[b] ±0.44	1.72 ±0.43	1.44 ±0.51	1.32 ±0.30	1.66 ±0.53	2.52[c] ±0.37
		0.82 ± 0.11									

[a] $p < .0025$ for paired values (0 vs 20 min).

[b] $p < .05$ against pooled basal values for PDV IRG.

[c] $p < .05$ against 60-min value for PDV IRG.

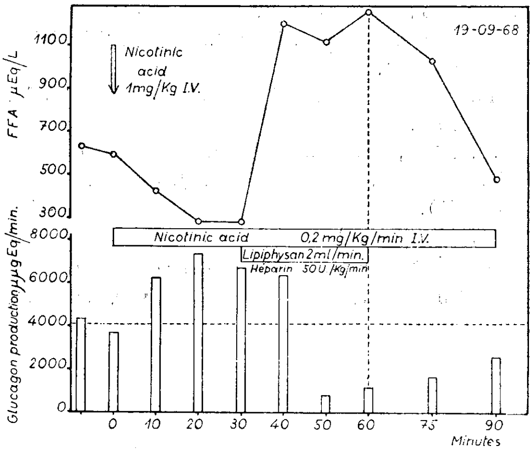

FIG. 2. Changes in pancreatic glucagon production related to changes in arterial plasma FFA concentrations.

tween the curves of PDV glucagon and arterial FFA, it seems reasonable to regard the glucagon response as induced by the changes in FFA concentrations. Although a direct effect of nicotinic acid on glucagon secretion can not be excluded, it seems less likely. A rise in PDV glucagon during hypoglycemia has been demonstrated in dogs by Unger *et al.* (2) and by Vance *et al.* (4). In our experiments, only negligible and inconstant variations in blood glucose were observed. Similarly, since there was no significant change in plasma amino nitrogen during the fall in plasma FFA, we can conclude that the glucagon response was not mediated by a rise in amino acids plasma concentration. Relative decrease in blood flow through the pancreas without modification of glucagon production could possibly account for the experimental findings but are ruled out by the simultaneous determination of arterial and PDV glucagon levels, PDV hematocrit, and measurement of the blood flow through the pancreas. It is possible to say, therefore, that in these experiments pancreatic glucagon production was actually increased in response to the fall in FFA level induced by nicotinic acid (Fig. 2). It has been recently established that circulating glucagon-like immunoreactivity is the product of several substances among which are two polypeptides factors originating from the digestive tract (20). Extracts of jejunal mucosa partially cross react with most but not all antisera to pancreatic glucagon (21). In the type of experiments here reported, comparison has been made between arterial and PDV plasma concentrations, and the differences observed can be attributed exclusively to changes in pancreatic glucagon production; the production of gut glucagon-like immunoreactivity has not yet been tested.

Summary. Artificial changes in plasma FFA concentrations were induced in dogs by infusion of nicotinic acid and of triglycerides with added heparin. In all experiments, the fall in plasma FFA observed during the nicotinic acid infusion or after terminating the TG-heparin infusion was accompanied by an increase in pancreaticoduodenal venous plasma glucagon concentrations corresponding to a true increase in pancreatic glucagon production. These results support the concept of an important role of plasma FFA in controlling the pancreatic glucagon production, at least in dogs.

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