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Effect of Oleic Acid on Insulin Secretion by the Isolated Perfused Rat Pancreas*

J. E. Campillo, A. S. Luyckx, M. D. Torres, and P. J. Lefebvre

Division of Diabetes, Institute of Medicine, University of Liège, Liège, Belgium

Summary. The isolated perfused rat pancreas was utilized to investigate the effect of oleic acid on insulin secretion. In the absence of glucose, a continuous infusion of oleic acid (1500 μmol/l) induced a biphasic insulin release. This effect was reduced at low extracellular calcium concentration. In the presence of oleic acid 1500 μmol/l, the insulin response to 10 mmol/l arginine occurrred earlier, the total amount of insulin released in response to the amino acid being unchanged. Such an effect was not obtained when oleic acid in the medium was 750 μmol/l, but it was observed in the presence of oleic acid 1500 μmol/l when the concentration of albumin in the perfusate was increased from 2 g/100 ml to 4 g/100 ml. The insulin response to a continuous infusion of glucose (4.4 mmol/l and 16.7 mmol/l) was potentiated by the presence of oleic acid 1500 μmol/l in the perfusate. No modification of the biphasic pattern of insulin response to glucose 16.7 mmol/l was observed. These results demonstrate that high concentrations of oleic acid stimulate insulin release from the isolated perfused rat pancreas and modulate the insulin response to arginine or glucose.

Key words: Arginine, glucose, insulin, isolated perfused rat pancreas, oleic acid, non-esterified fatty acid.

The important role of insulin in the control of fatty acid metabolism is well accepted [1]. In contrast, the effect of these substrates in the regulation of insulin release from the pancreatic beta-cells remains controversial. In fact, studies on the influence of non-esterified fatty acids (NEFA) on insulin secretion have yielded contradictory results in vivo both in humans [2–6] and in animals [7–13]. The in vitro studies of the effect of NEFA on insulin release are also uncertain [14–22].

The present work was undertaken to further investigate the effect of oleic acid on insulin release using the isolated perfused rat pancreas. The studies performed included the effects of oleic acid on basal insulin release and the effect of oleic acid in the perfusion medium on the insulin response to arginine or glucose.

Material and Methods

Male Wistar rats, weighing 250–300 g and, fed *ad libitum* with commercially available food, were utilized in all experiments. After on overnight fast, the animals were anesthetised with sodium pentobarbital (45 mg/kg body weight Nembutal Abbott) given intraperitoneally. Pancreases were isolated as previously described by Sussmann et al. [23] with minor modifications [24, 25]. The preparation consisted of the rat pancreas without the stomach and spleen and including only a small part of the duodenum. After removal, the preparation was immediately transferred to the perfusion chamber of an Ambec Perfusion Apparatus (Beck Industries, Boulder, Colorado, U.S.A.) and immersed in a bath of NaCl, 9 g/100 ml maintained at a constant temperature of 38 °C. The pancreas was perfused via a cannula inserted into the aorta, the effluent being collected from the cannulated portal vein without recycling. A cannula was inserted into the duodenum to allow evacuation of secretions. The flow rate was maintained constant at 2 ml/min which resulted in a perfusion pressure of 20–40 mm Hg.

The perfusate was a modified Krebs-Henseleit bicarbonate buffer containing the following components: NaCl 120 mmol/l, KCl 4 mmol/l, KH_2PO_4 1.2 mmol/l, $MgSO_4$ 0.7 mmol/l, $NaHCO_3$ 25 mmol/l and $CaCl_2$ at concentrations of 1.0 or 2.5 mmol/l. The medium was supplemented with 2 g/100 ml bovine crystallised albumin (Armour Pharmaceutical Co, Eastbourne England) and 2.5 g/100 ml dextran (MW 70000 Poviet Christiaens, Bruxelles,

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Table 1. NEFA/Albumin ratios in the various experimental conditions

Incubation medium	Oleic acid addition (μmol/l)	NEFA measured (μmol/l)	mol NEFA/mol Albumin
Standard:			
Albumin 2 g/100 ml	Nil	346±15	1.19
Dextran 2.5 g/100 ml		(n=25)	
Ditto	1500	1632±39	5.63
		(n=15)	
Ditto	750	1032±126	3.56
		(n=3)	
Modified:			
Albumin 4 g/100 ml	Nil	633±55	1.09
Dextran 0.5 g/100 ml		(n=4)	
Ditto	1500	1728±84	2.98
		(n=3)	

[a] Assuming M. W. 69.000

Belgium). Unless otherwise stated, this medium ("standard medium") has been used in all experiments. In some experiments, the albumin concentration was raised to 4 g/100 ml and the dextran concentration reduced to 0.5 g/100 ml. The medium containing oleic acid was prepared by adding oleic acid (Merck, Darmstadt, Germany) to a Krebs bicarbonate buffer at 38 °C (without $CaCl_2$ which was added afterwards) and then agitating the mixture for 30 min with a magnetic stirrer. The medium was continuously gassed with a mixture of O_2 and CO_2 (95:5); the resulting pH was 7.5.

The following experiments were performed:

1. Effect of Oleic Acid on Insulin Secretion: after removal, the pancreas was perfused for 30 min with the standard medium; it was then substituted by a medium containing oleic acid (1500 μmol/l) infused from a reservoir via three way valves which allowed rapid changes without affecting flow rate.

2. Effect of Oleic Acid on the Insulin Response to Arginine or Glucose: the pancreas was perfused from the beginning of the experiments using a medium with or without added oleic acid for 30 min before exposure to the secretatogues. Arginine (L-arginine HCl, Fluka A.G., Buchs, Switzerland) and glucose (Merck) were dissolved in the perfusate and infused into the circuit immediately above the pancreas through a side-arm perfusion pump working at a flow rate of 0.1 ml/min. Control experiments without infusing glucose or arginine were also performed, with or without oleic acid present in the medium from the beginning of the perfusion.

Perfusate samples (2 ml) were collected from the cannula inserted in the portal vein into chilled tubes, containing 1000 U Trasylol (Bayer, Leverkusen, Germany) and immediately frozen for storage at −20° C until assayed. The final fatty acid concentration reached in each experiment was measured according to Dole and Meinertz [26]. Table 1 indicates the concentrations of NEFA measured in the various experimental conditions and the respective values of the FFA/albumin ratios. Insulin (immunoreactive insulin, IRI) was measured in duplicate by the double antibody procedure of Hales and Randle [27] with rat insulin as standard (Novo, Copenhagen, Denmark). Insulin dilutions for standard curves were performed in the perfusion medium, with or without oleic acid, to obtain the same conditions as in the sample tubes. The readings were made on the respective curves for the samples containing or not oleic acid. Oleic acid did not interfere with the immunoassay since no difference between insulin standard curves due to the presence of oleic acid was detected. Insulin secretion rates were calculated by multiplying the concentration in the respective samples by the flow rate and expressed as ng/min. Total release during the stimulation period were obtained by planimetry of the individual perfusion profiles and by calculating the mean of the respective areas. All results are expressed as mean ± standard error of the mean (SEM). Statistical evaluation was performed using the Student *t* test for non paired data.

Results

1. Effect of Oleic Acid Alone on Insulin Release

Figure 1 shows the effect of a continuous infusion of oleic acid (1500 μmol/l) on insulin release in the absence of glucose in the perfusate. A biphasic insulin response was clearly observed when the perfusate contained 2.5 mmol/l calcium. The peak response for the first phase always occurred within two minutes. The secondary rise consisted of a more gradual increase in the insulin secretion rate which reached a maximum at 20 minutes, and then spontaneously declined while remaining higher than the basal level. At a lower calcium concentration (1.0 mmol/l) in the perfusate, both the first and the second phases of the insulin response to oleic acid were significantly reduced. The total amount of insulin released in response to the 60 minute oleic acid infusion was 18.0 ± 2.5 ng/60 min at 1.0 mmol/l calcium and 65.1 ± 12.7 ng/60 min at 2.5 mmol/l calcium ($p < 0.02$).

2. Effect of Oleic Acid on the Arginine-Induced Insulin Release

The pancreas was perfused with the standard medium or with the 1500 μmol/l oleic acid-enriched medium from the beginning of the experiment and insulin release measured between 30 and 75 min from the start of the perfusion. Basal insulin release, at 2.5 mmol/l calcium, was slightly but not significantly (N.S.) higher in the presence (0.52 ± 0.18 ng/min) than in the absence (0.10 ± 0.02 ng/min) of oleic acid. At 1.0 mmol/l calcium, basal insulin release was 0.75 ± 0.26 ng/min in the presence and 0.19 ± 0.03 ng/min in the absence of oleic acid (N.S.).

In the absence of glucose and oleic acid in the perfusate, arginine (10 mmol/l) elicited a monophasic rise in insulin release (Figures 2, 3 and 4). As shown by Figure 2, the arginine-induced insulin release was not modified by the presence of 750 μmol/l oleic acid in the perfusate. Total insulin release during the stimulation period was 126.64 ± 13.7 ng/30 min and 134.9 ± 16.9 ng/30 min, in the

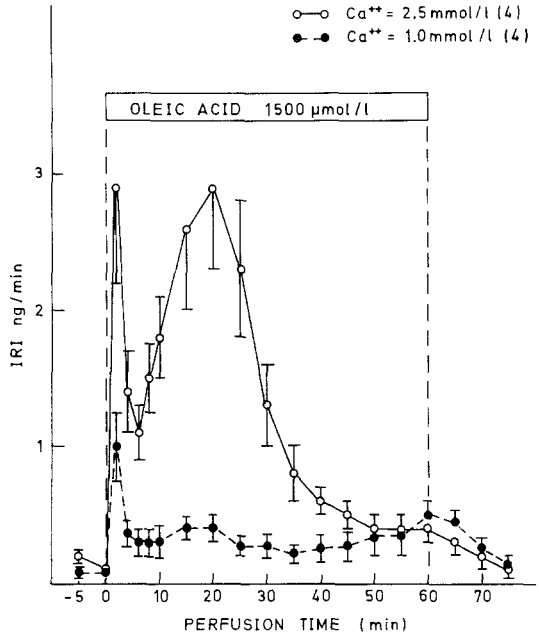

Fig. 1. Oleic acid-induced insulin (IRI) release by the isolated perfused rat pancreas in a glucose-free perfusate. The pancreas was perfused for 30 min before the beginning of the oleic acid infusion. The number of perfusions is indicated in parentheses. Results are given as mean ± SEM

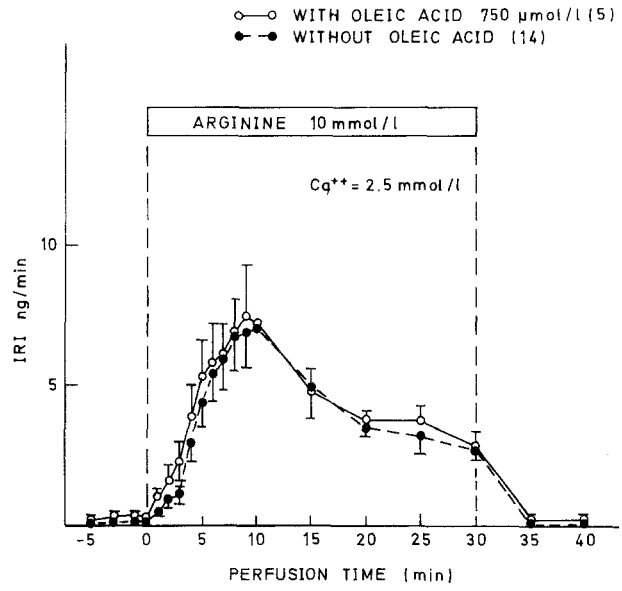

Fig. 2. Influence of 750 µmol/l oleic acid in the perfusate from the beginning of the perfusion on the arginine-induced insulin (IRI) release. No glucose was present in the medium. The number of perfusions is indicated in parentheses. Results are given as mean ± SEM

absence and in the presence of oleic acid respectively (N.S.). In contrast (Fig. 3), when 1500 µmol/l oleic acid was present in the perfusate, a monophasic pattern of arginine-induced insulin release was also observed, but the hormonal response occurred significantly earlier without modification of the total amount of insulin released in response to the amino acid. At 1.0 mmol/l calcium, the amount of insulin released during the stimulatory period was 61.78 ± 10.3 ng/30 min and 58.5 ± 8.2 ng/30 min in the absence and presence of oleic acid respectively (N.S.). At 2.5 mmol/l calcium, the amount of insulin released during the same period averaged 125.64 ± 13.7 ng/30 min and 162.2 ± 21.7 ng/30 min in the absence and presence of oleic acid respectively (N.S.). A significantly greater insulin response to the amino acid was observed at 2.5 mmol/l calcium both in the absence ($p < 0.01$) and in the presence ($p < 0.001$) of 1500 µmol/l oleic acid in the perfusate.

In addition and as shown in Figure 4, the earlier insulin response to arginine induced by oleic acid (1500 µmol/l) was also observed when the perfusion medium contained 4 g/100 ml albumin. In these conditions, total insulin released during the stimulatory period was 99.9 ± 22.9 ng/30 min and 145.8 ± 28.0 ng/30 min in the absence and presence of oleic acid respectively (N.S.).

3. Effect of Oleic Acid on the Glucose-Induced Insulin Release

Figure 5 shows the effect of oleic acid (1500 µmol/l) in the perfusate on the insulin response to a low glucose concentration (4.4 mmol/l). In these experiments, oleic acid was infused from the beginning of the perfusion and no glucose was present in the medium during the prestimulatory period. In the absence of oleic acid, only a shortlived modest first peak insulin response was observed; it was significantly ($p < 0.05$) enhanced by the presence of oleic acid. The total amount of insulin released during the stimulatory period averaged 7.99 ± 3.0 ng/30 min in the absence and 22.8 ± 3.4 ng/30 min in the presence of oleic acid ($p < 0.05$). Thus oleic acid being present in the perfusate potentiated the insulin response to 4.4 mmol/l glucose. When 16.7 mmol/l glucose was used as secretagogue (Figure 6), both in the presence and in the absence of oleic acid a biphasic pattern of insulin response was observed. Total insulin released during the stimulatory period averaged 130.5 ± 22.2 ng/30 min in the absence and 349.5 ± 63.5 ng/30 min in the presence of oleic acid ($p < 0.05$). Considerable potentiation of the 16.7 mmol/l glucose-induced insulin release by oleic acid was thus observed.

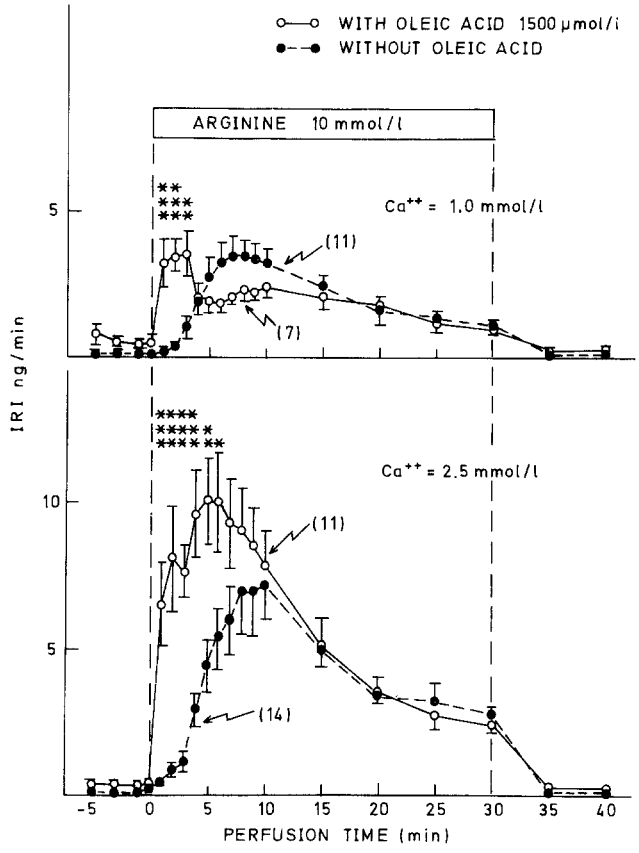

Fig. 3. Influence of 1500 µmol/l oleic acid on the arginine-induced insulin (IRI) secretion in a glucose free perfusate. Oleic acid was present in the medium from the beginning of the perfusion. The number of perfusions is indicated in parentheses. Results are given as mean ± SEM. Statistical comparisons are: * $p < 0.01$; ** $p < 0.005$; *** $p < 0.001$

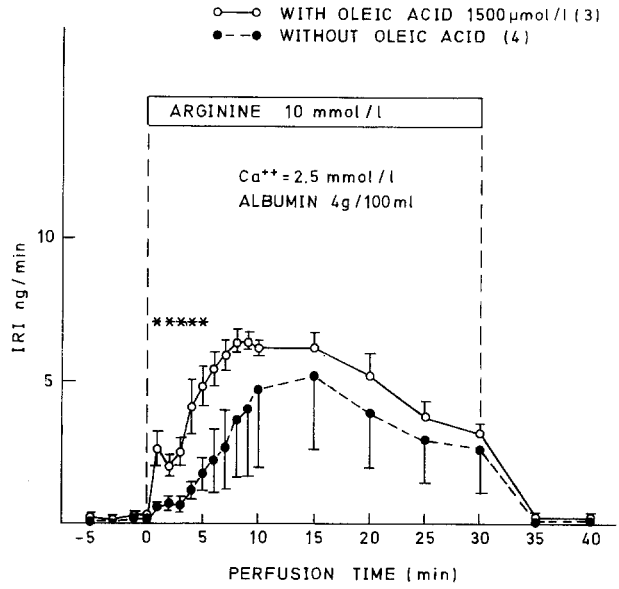

Fig. 4. Influence of 1500 µmol/l oleic acid on the arginine-induced insulin (IRI) release in a glucose free perfusate containing 4 g/100 ml albumin. Oleic acid was present in the medium from the beginning of the perfusion. The number of perfusions is indicated in parentheses. Results are given as mean ± SEM. Statistical comparisons is: * $p < 0.01$

Discussion

The role of fatty acids in the regulation of insulin secretion is not yet clearly defined. In man, the effect of NEFA on insulin secretion has been studied by giving oral or intravenous fat accompanied by heparin injection. Schalch et al. [2] and Balasse et al. [5] found that elevation of plasma NEFA by fat ingestion and intravenous heparin did not significantly affect basal plasma insulin in man. In contrast, Raptis et al. [5] found a significant rise in insulin secretion after both intravenous and oral administration of fat. Hicks et al. [6] reported that, during elevation of NEFA plasma levels by infusion of a triglyceride emulsion supplemented with heparin, the mean basal level of plasma insulin increased by 100%. In dogs, the acute elevation of plasma NEFA levels by infusion of triglyceride emulsion and heparin stimulated insulin secretion [8, 10]. Infusion of sodium salts of oleic, linoleic, lauric or palmitic acid into the pancreatic artery of anesthetized dogs, produced a biphasic insulin response [11, 12]. In fasted rats, infusion of oleic acid emulsion stabilized with albumin doubled the plasma insulin concentrations [13].

The in vitro effects of NEFA on insulin release are also conflicting. Howell [18], in rat pancreas slices, and Pi-Sunyer [20], in pieces of rat pancreas, were unable to demonstrate any effect of palmitate, octanoate or oleate on insulin release at either low or high glucose concentration. Such negative results were also reported recently by Pek et al. [21] who did not observe any change in the insulin secretion rate in response to a one minute oleic acid (10 µmol/l) infusion in the isolated perfused rat pancreas. In contrast, Montague et al. [17], in isolated rat islets, and Sanbar et al. [15], in pieces of rat pancreas, reported that octanoate directly stimulated insulin release. Malaisse et al. [16], using incubated pieces or rat

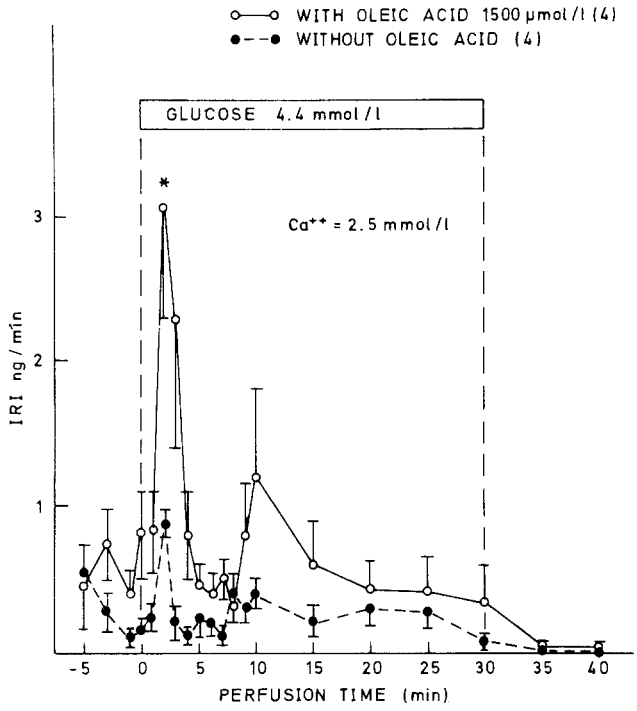

Fig. 5. Influence of 1500 µmol/l oleic acid on the insulin (IRI) response to 4.4 mol/l glucose. Oleic acid was present from the beginning of the perfusion. No glucose was present in the perfusate during the pre-stimulatory period. The number of perfusions is indicated in parentheses. Results are given as mean ± SEM. Statistical comparisons is: * $p < 0.05$

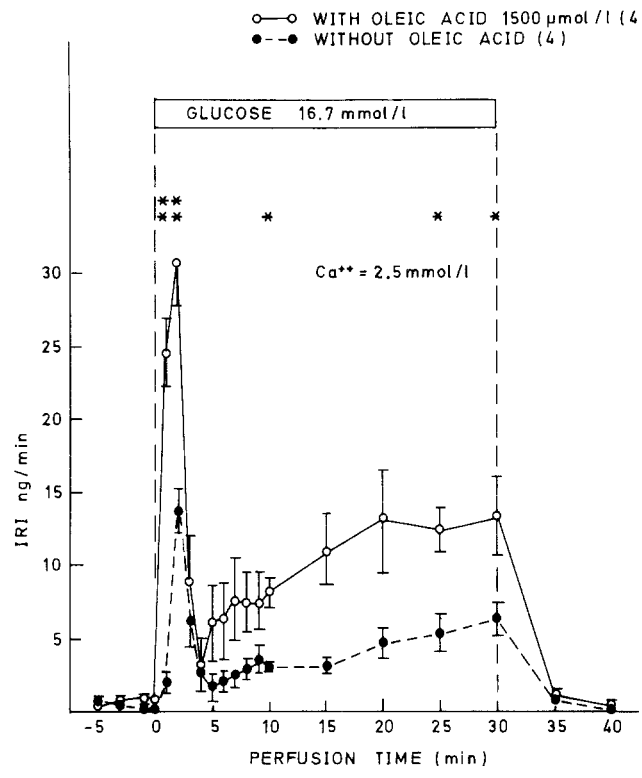

Fig. 6. Influence of oleic acid on the insulin (IRI) response to the infusion of 16.7 mmol/l glucose. Oleic acid was present from the beginning of the perfusion. No glucose was present in the perfusate during the pre-stimulatory period. The number of perfusions is indicated in parentheses. Results are given as mean ± SEM. Statistical comparisons are: * $p < 0.05$; ** $p < 0.01$

pancreas found that insulin secretion was stimulated by β-hydroxybutyrate and palmitate but not by octanoate. Using the isolated perfused rat pancreas, Goberna et al. [19] reported that neither palmitate, β-hydroxybutyrate nor acetoacetate were capable of increasing insulin release when perfused in the absence of glucose.

Since studies in vitro have often failed to demonstrate any effect of long-chain fatty acids on insulin release in the absence of extracellular glucose [14, 16, 19), it has been suggested that the stimulatory effect of NEFA on insulin secretion was glucose-dependent and that minimum glucose levels were necessary to "prime" beta-cells [19].

In the present experiments, a continuous infusion of oleic acid in the isolated rat pancreas clearly induced a biphasic insulin release in the absence of extracellular glucose. This effect was significantly reduced at low calcium concentration. The insulin response to oleic acid infusion was rapid: maximal rates of insulin release were attained within two minutes after the beginning of the infusion. It is noteworthy that the rate of insulin release declined to a lower level after the secondary rise despite further continuing infusion of oleic acid. This effect agrees with the results reported by Goberna et al. [19] in the isolated perfused rat pancreas and by Crespin et al. [12] in anesthetized dogs. This kinetic pattern is in striking contrast to the well established sustained secondary phase of glucose-induced insulin release [28]; it is more similar to what has been reported for arginine [29, 31]. These data seem to indicate that high concentrations of oleic acid as well as arginine can stimulate insulin release in the absence of glucose, but that the effect is of short duration. Its biological significance remains to be elucidated. The evanescent character of the oleic acid-induced insulin secretion in the isolated perfused pancreas probably explains why it has not been observed [18, 19] when isolated pieces of pancreas were utilized.

Both oleic acid- and arginine-induced insulin release, in a glucose-free medium, are influenced by the extracellular calcium concentration. Unlike results obtained at 1.0 mmol/l calcium, when the

extracellular calcium concentration was 2.5 mmol/l a significant increase of the insulin response to oleic acid or arginine was observed, without modification of the respective patterns of release.

The mechanism of the insulin releasing effect of fatty acids and ketone bodies is so far obscure. It has been shown recently that both medium-chain and long-chain fatty acids and ketone bodies are oxidized by the beta-cell [32]. The oxidation rates found for these substrates were of such a magnitude that they can be assumed to represent an important part of the total islet energy metabolism. However, as was the case with amino acids [32], a direct correlation between the rate of oxidation of fatty acids and their insulin-releasing capacity is difficult to establish.

The present results also show that, aside from the stimulating effect that oleic alone exerts on insulin secretion, it also modulates the arginine- and glucose-induced insulin release. When oleic acid (1500 μmol/l) was present in the medium, the insulin response to arginine occurred clearly earlier without modification of the total amount of insulin released. In contrast, the total amount of insulin released in response to glucose was significantly increased in the presence of oleic acid without modification of the biphasic pattern of release.

Little attention has been paid to the effect of fatty acids or ketone bodies on the insulin response to glucose or amino acids [6, 19]. The effects of oleic acid (more rapid insulin response to arginine and potentiation of the response to glucose) are similar to those elicited by the presence of a basal, non-stimulatory, glucose concentration [28, 34]. Low glucose or high oleic acid could act by a similar mechanism on the beta-cell, i. e. providing sufficient energy to facilitate insulin release mechanisms to take place.

Many problems are involved in the in vitro experiments using long chain fatty acids. One such problem is the level of albumin that is present in the medium. Under normal physiological conditions, most on the fatty acids are bound to albumin and each molecule of albumin, on an average, has only 7 sites for binding NEFA [35]. In our experimental conditions, the albumin concentration present in the perfusate allowed binding all the oleic acid added. On the other hand, it is known that NEFA exceeding the transport capacity of albumin could exert profound unphysiological effects on cells. In our hands however, when the effect of oleic acid (1500 μmol/l) on the arginine-induced insulin release was tested in the presence of 4 g/100 ml albumin the results obtained were similar to those seen using 2 g/100 ml albumin. This seems to rule out the possibility that the effects observed are due to NEFA exceeding the binding capacity of albumin. The effect reported, however, could perhaps be due not to oleic acid specifically since oleic acid might displace other FFA from albumin, albeit bound to high affinity sites.

In conclusion, the results clearly show that oleic acid may play a role in the modulation of insulin secretion by the isolated perfused rat pancreas. Whether this role is physiologically relevant remains to be assessed.

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Dr. A. S. Luyckx is Maître de Recherches du Fonds National de la Recherche Scientifique (Belgium). Dr. J. E. Campillo is a visiting scientist whose permanent addres is: Departamento de Fisiologia, Faculdad da Medicina, Universidad de Granada, Granada, Spain.

References

1. Avruch, J., Carter, J. P., Martin, D. B.: The effect of insulin on the metabolism of the adipose tissue. In: Handbook of Physiology. Steiner, D. F., Freinkel, N. (Eds), Section 7: Endocrinology, Volume I: Endocrine Pancreas, p. 545–562. Washington: American Physiological Society 1972
2. Schalch, D. S., Kipnis, D. M.: Abnormalities in carbohydrate tolerance associated with elevated plasma non esterified fatty acids. J. Clin. Invest. **44**, 2010–2020 (1965)
3. Balasse, E., Ooms, H. A.: Effet d'une élévation aigue due taux des acids gras libres (NEFA) sur la tolérance glucidique et la réponse insulinique à l'hyperglycémie chez l'homme normal. Rev. Fr. Et. Clin. Biol. **13**, 62–67 (1968)
4. Pi-Sunyer, F. X., Hashim, S. A., Van Itallie, T. B.: Insulin and ketone response to ingestion of medium and long-chain fatty acids in man. Diabetes **18**, 96–100 (1969)
5. Raptis, E. F., Kissing, J., Dollinger, H., Schroeder, K. E., Pfeiffer, E. F.: The effect of oral and intravenous administration of fat on the function of the endocrine and exocrine pancreas in man. Excerpta Med. Int. Congr. Series **280**, 37 (1973)
6. Hicks, B. H., Taylor, C. I., Vij, S. K., Pek, S., Knopf, R. F., Floyd, J. C., Fajans, S. S.: Effect of changes in plasma levels of free fatty acids on plasma glucagon, insulin and growth hormone in man. Metabolism **26**, 1011–1023 (1977)
7. Sanbar, S. S., Evans, J. R., Heteni, G., Jr.: Further studies on the effect of octanoate on glucose metabolism in dogs. Can. J. Physiol. Pharmacol. **45**, 29–33 (1967)
8. Seyffert, W. A., Madison, L. L.: Physiologic effects of metabolic fuels on carbohydrate metabolism. I. Acute effect of elevation of plasma free fatty acids on hepatic glucose output, peripheral glucose utilization, serum insulin and plasma glucose levels. Diabetes **16**, 765–776 (1967)
9. Manns, J. G., Boda, J. M., Willes, R. F.: Probable role of propionate and butyrate in control of insulin secretion in sheep. Am. J. Physiol. **212**, 756–764 (1967)
10. Madison, L. L., Seyffert, W. A., Unger, R. H., Barker, B.: Effect of plasma free fatty acids on plasma glucagon and serum insulin concentration. Metabolism **17**, 301–304 (1968)
11. Crespin, S. R., Greenough III, W. B., Steinberg, D.: Stimulation of insulin secretion by infusion of free fatty acids. J. Clin. Invest. **48**, 1934–1943 (1969)
12. Crespin, S. R., Greenough, W. B., Steinberg, D.: Stimulation of insulin secretion by infusion of long-chain free fatty acids. A direct pancreatic effect. J. Clin. Invest. **52**, 1979–1984 (1973)

13. Bates, M. W., Linn, L. C., Huen, A. H.: Effects of oleic acid infusion on plasma free fatty acids and ketone bodies in the fasting rat. Metabolism **25**, 361–373 (1976)
14. Coore, H. G., Randle, P. J.: Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J. **93**, 66–78 (1964)
15. Sanbar, S. S., Martin, J. M.: Stimulation by octanoate of insulin release from isolated rat pancreas. Metabolism **16**, 482–484 (1967)
16. Malaisse, W. J., Malaisse-Lagae, F.: Stimulation of insulin secretion by noncarbohydrate metabolites. J. Lab. Clin. Med. **72**, 438–448 (1968)
17. Montague, W., Taylor, K. N.: Regulation of insulin secretion by short chain fatty acids. Nature **217**, 853 (1968)
18. Howell, S. L.: Thesis, Univ. London (1967)
19. Goberna, R., Tamarit, J. Jr., Osorio, J., Fussganger, R., Tamarit, J., Pfeiffer, E. F.: Action of B-hydroxybutyrate, acetoacetate and palmitate on the insulin release in the perfused isolated rat pancreas. Horm. Metab. Res. **6**, 256–260 (1974)
20. Pi-Sunyer, F. X.: Effect of long and medium chain fatty acids on insulin secretion from pieces of rat pancreas. Proc. Soc. Exp. Biol. Med. **149**, 693–697 (1975)
21. Pek, S., Tong-Yuan, T., Elster, A.: Stimulatory effects of prostaglandins E-1, E-2, and F-2-alpha on glucagon and insulin release in vitro. Diabetes **27**, 801–809 (1978)
22. Jordan, H. N., Phillips, R. W.: Effect of fatty acids on isolated ovine pancreatic islets. Am. J. Physiol. **234**, 162–167 (1978)
23. Sussman, K. E., Vaughan, G. D., Timmer, R. F.: An in vitro method for studying insulin secretion in the perfused isolated rat pancreas. Metabolism **15**, 466–476 (1966)
24. Luyckx, A. S., Lefebvre, P. J.: Changes in insulin and glucagon secretion related to concentrations of metabolic substrates in the isolated perfused pancreas. In: Hormones pancréatiques, Hormones de l'eau et des électrolytes. pp. 99–127. Paris: Inserm 1972
25. Fussganger, R. D., Straub, K., Goberna, R., Jaros, P., Schroeder, K. E., Raptis, S., Pfeiffer, E. F.: Primary secretion of insulin and secondary release of glucagon from the isolated perfused rat pancreas following with pancreozymin. Horm. Metab. Res. [Suppl.] **1**, 224–227 (1969)
26. Dole, V. P., Meinertz, H.: Microdetermination of long chain fatty acids in plasma. J. Biol. Chem. **235**, 2395–2399 (1960)
27. Hales, C. N., Randle, P. J.: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. **88**, 137–146 (1963)
28. Grodsky, G. M.: Secretion of insulin. The kinetics of insulin release. In: Handbook of Experimental Pharmacology XXXII/2. Hasselblatt, A., Bruchhausen, F. V. (Eds), pp. 1–16. Berlin, Heidelberg, New York: Springer 1975
29. Gerich, J. E., Charles, A., Grodsky, G. M.: Characterization of the effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas. J. Clin. Invest. **54**, 833–841 (1974)
30. Gerich, J. E., Frankel, B. J., Franska, R., West, L., Forsham, P. H., Grodsky, G. M.: Calcium dependency of glucagon secretion from the in vitro perfused rat pancreas. Endocrinology **94**, 1381–1385 (1974)
31. Campillo, J. E., Luyckx, A. S., Torres, M. D., Lefebvre, P. J.: Effect of various concentrations of calcium on arginine-induced insulin and glucagon release in vitro. Rev. Esp. Fisiol. **34**, 191–198 (1978)
32. Berne, C.: The metabolism of lipids in mouse pancreatic islets. Biochem. J. **152**, 661–666 (1975)
33. Hellman, B., Sehlin, J., Taljedal, I. B.: Effects of glucose and other modifiers of insulin release on the oxidative metabolism of amino acids in micro-dissected pancreatic islets. Biochem. J. **123**, 513–521 (1971)
34. Efendic, S., Cerasi, E., Luft, R., Gladnikoff, G.: Potentiation of glucose-induced insulin release by glucose in the isolated pancreas of fed and fasted rats. Diabetes **25**, 949–954 (1976)
35. Spector, A. A.: Fatty acid binding to plasma albumin. J. Lipid Res. **16**, 165–179 (1975)

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Dr. P. J. Lefebvre
Division of Diabetes
Institute of Medicine
Hôpital de Bavière
Blv. de la Constitution, 66
B-4020 Liège
Belgium