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Effect of Long- and Medium-Chain Fatty Acids on Insulin Secretion from Pieces of Rat Pancreas[1] (38881)

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Free fatty acids (FFA) and ketone bodies are utilized for energy in the starved state, replacing carbohydrate as the major fuel (1). It has been postulated that during starvation both FFA (2) and ketones (3) have an important influence in modulating lipolysis by enhancing insulin secretion, thereby ensuring a safe transition from carbohydrate to fat metabolism without the danger of uninhibited lipolysis and ketoacidosis. However, there are contradictory reports about the effect of FFA on insulin secretion *in vitro* and *in vivo*. Because of these discrepant results, a series of *in vitro* experiments have been done to evaluate the effect of long-chain (oleate) and of medium-chain (octanoate) fatty acids on insulin secretory response from pieces of rat pancreas.

Materials and Methods. Animals. Male Sprague–Dawley rats were maintained on standard laboratory chow. On the day of the experiment, the animals (150–250 g) were anesthetized with sodium pentobarbital (4 mg per 100 g), the pancreas was quickly dissected free, placed in saline in a petri dish, and all visible fat was trimmed away. Slices weighing about 100 mg were then cut and placed into 25-ml incubation flasks.

Incubation media. The medium was Krebs–Ringer–bicarbonate buffer (4), supplemented in most experiments with sodium pyruvate,[2] sodium glutamate,[2] and sodium fumarate[3] at a concentration of 5 m*M* each. Fatty acid-free albumin[4] was prepared by the method of Chen (5) and used at a concentration of 0.2 mg/ml for the octanoate experiments and 40 mg/ml for the oleate experiments. Glucose was added at a concentration of 0.5 or 3.0 mg/ml. The fatty acids, sodium octanoate and sodium oleate, were added as indicated below. Sodium oleate was solubilized as follows (6): oleic acid[2] (50 mmoles) was mixed with 1 ml of 0.1 *N* sodium hydroxide and added to 24 ml of fatty-acid free albumin (8.0 g w/v) at pH 7.4. The mixture was stirred for at least 15 hr and had to be clear before use. To prepare the incubation medium, 1 vol of this mixture was added to 1 vol of a solution containing suitable amounts of the normal constituents of the medium. The incubation was carried out in a Dubnoff shaker at 37° under 95 % O_2–5 % CO_2.

Incubation pattern. In one series of experiments, carried out according to the method of Coore and Randle (7), the pancreas pieces were preincubated for 30 min in 20 ml of medium (0.6 mg/ml glucose), washed with fresh medium, and taken through a series of four additional 15-min incubations in separate vessels. For this purpose, one piece of tissue was incubated with 2 ml of a medium containing glucose at concentrations of either 0.6 mg/ml or 3.0 mg/ml with or without sodium octanoate (3 m*M*) or sodium oleate (1 m*M*). A series of experiments were also done with oleate at a 2 m*M* concentration. This required that the albumin concentration be raised to 80 mg/ml. To rule out the possibility that pyruvate, glutamate, and fumarate may stimulate the pancreas maximally and preempt further response to the added fatty acid, other pancreatic slices were incubated under identical conditions, but without these agents.

Before each 15-min incubation, the pancreatic piece was rinsed for 1 min in a small quantity of the medium in which it was to be incubated. Between the second and the third incubation periods, the tissue was allowed to "rest" for 30 min at low glucose concentration (0.6 mg/ml). The 15-min period was chosen to prevent significant degradation of

[1] Supported in part by NIH Grant AM17624.

[2] Matheson Coleman and Bell.

[3] K & K Laboratories.

[4] BVO 362, Pentex, Inc.

TABLE I. INSULIN SECRETION FROM RAT PANCREAS PIECES INCUBATED FOR 15 MIN WITH AND WITHOUT FATTY ACID IN THE PRESENCE OF PYRUVATE, SUCCINATE, AND FUMARATE.[a]

	Octanoate			Oleate					
	0	3 mM	*P*	0	1 mM	*P*	0	2 mM	*P*
Glucose (mg/ml)									
0.6	4.5 ±0.6	5.9 ±1.2	NS	5.1 ±0.9	6.7 ±1.0	NS	14.7 ±4.1	23.4 ±8.6	NS
3.0	26.4 ±3.2	26.4 ±3.4	NS	23.4 ±3.7	33.4 ±5.5	NS	50.9 ±10.7	52.4 ±10.8	NS
P	<0.001	<0.001		<0.001	<0.001		<0.001	<0.025	
			(*n* = 42)			(*n* = 23)			(*n* = 16)

[a]Insulin is expressed as nanograms per minute per gram wet weight pancreas.

insulin by proteolytic enzymes leached from the pancreas (7) and possible inhibition of insulin secretion by the insulin already secreted into the medium (8). The period, however, was long enough to allow significant insulin secretion in response to a variety of secretagogues including glucose (7) and FFA (9).

Forty-two pieces of pancreas from five rats were used in 168 incubations with octanoate (42 in each of four media), while 23 pieces from three rats were used in 92 incubations with 1 m*M* oleate (23 in each of four media) and 16 pieces from three rats in 48 incubations with 2 m*M* (16 in each of the four media). Eight pieces from two rats were used in 32 incubations with 1 m*M* oleate and without pyruvate, glutamate, and fumarate in the medium (eight in each of the four media).

In another series of experiments the tissue samples were incubated for 90 min in order to detect any insulin secretory effect of fatty acids which might have been missed in the shorter experiments. The pancreas pieces were preincubated for 30 min in 20 ml of KRB medium (0.6 mg/ml glucose), washed with fresh preincubation medium, and incubated in 2 ml of appropriate medium for 90 min. To inhibit degradation of insulin by proteolytic enzymes leached from the pancreas, a Kallikrein inhibitor (trasylol, 625 kIU/ml) was added to the incubation medium (10).

In each 90-min experiment we used 24 pieces of pancreas, eight taken from each of three rats. These were distributed so that two pieces from each rat were incubated in each of the four media: high (3.0 mg/ml) and low (0.6 mg/ml) glucose, with and without fatty acid. Seventy-two pieces from nine rats were used in the octanoate experiments (18 pieces from nine rats in each of the four media) and 72 pieces from nine rats in each of the two oleate experiments (1 m*M* and 2 m*M*). Additional 90-min incubations were done without pyruvate, succinate, and fumarate. Again, 72 pieces from nine rats were used for testing the octanoate and a similar number for oleate.

TABLE II. INSULIN SECRETION FROM RAT PANCREAS PIECES INCUBATED FOR 15 MINUTES WITH AND WITHOUT FATTY ACID, IN THE ABSENCE OF PYRUVATE, SUCCINATE, AND FUMARATE.[a]

	Oleate		
	0	1 mM	*P*
Glucose (mg/ml)			
0.6	13.0 ± 6.8	7.9 ± 2.8	NS
3.0	36.8 ± 14.5	21.4 ± 6.6	NS
P	<.025	<.005	(*n* = 8)

[a] Insulin is expressed as nanograms per minute per gram wet weight pancreas.

Analyses and calculations. Insulin concentrations in the incubation media were determined by a double-antibody immunoassay system (11), using a rat insulin standard.[5] For the short-incubation experiments, we calculated the significance between means of paired samples. For the long-incubation experiments, the significance between means of the four incubation media groups was cal-

[5] Novo Laboratories, Denmark.

TABLE III. INSULIN SECRETION FROM RAT PANCREAS PIECES INCUBATED FOR 90 MINUTES WITH AND WITHOUT FATTY ACID IN THE PRESENCE OF PYRUVATE, SUCCINATE, AND FUMARATE.[a]

	Octanoate			Oleate					
	0	3 m*M*	*P*	0	1 m*M*	*P*	0	2 m*M*	*P*
Glucose (mg/ml)									
0.6	3.42 ±0.59 (18)	2.92 ±0.42 (18)	NS	1.83 ±0.44 (18)	1.74 ±0.29 (18)	NS	1.69 ±0.28 (18)	1.81 ±0.32 (18)	NS
3.0	15.81 ±2.28 (18)	13.06 ±1.74 (18)	NS	11.08 ±1.50 (18)	11.36 ±1.30 (18)	NS	11.46 ±2.06 (18)	9.98 ±0.90 (18)	NS
P	<0.001	<0.001 (*n* = 72)		<0.001	<0.001 (*n* = 72)		<0.001	<0.001 (*n* = 72)	

[a] Insulin is expressed as nanograms per minute per gram wet weight pancreas.

TABLE IV. INSULIN SECRETION FROM RAT PANCREAS PIECES INCUBATED FOR 90 MINUTES WITH AND WITHOUT FATTY ACIDS.[a]

	Octanoate			Oleate		
	0	3 m*M*	*P*	0	2 m*M*	*P*
Glucose (mg/ml)						
0.6	3.64 ± 0.65 (18)	3.38 ± 0.49 (18)	NS	1.52 ± 0.22 (18)	1.40 ± 0.18 (18)	NS
3.0	6.49 ± 1.17 (18)	5.27 ± 0.71 (18)	NS	4.97 ± 0.70 (18)	6.19 ± 0.88 (18)	NS
P	<0.025	<0.05 (*n* = 72)		<0.001	<0.001 (*n* = 72)	

[a] Insulin is expressed as nanograms per minute per gram wet weight pancreas.

culated by Student's *t* test. Malaisse and Malaisse-Lagae (6) have reported a concentration-dependent reduction of insulin-binding capacity of anti-insulin serum by 2.0- to 40.0-m*M* sodium octanoate. Even though the final octanoate concentration in our individual assay tubes was 0.15 m*M* or less, we prepared a series of rat insulin standard curves with octanoate at concentrations of 0, 0.3, 0.6, and 1.0 m*M*. No difference between these insulin standard curves could be detected.

Results. The insulin secretory responses of the rat pancreas pieces obtained in the 15-min incubation experiments are given in Table I. No significant differences were observed after the addition of octanoate or oleate, at either glucose concentration. On the other hand, the marked response to high glucose concentration testifies to the viability of the tissue incubated.

Table II gives the insulin secretory response of rat pancreatic slices incubated for 15-min intervals in a medium that did not contain pyruvate, glutamate, and fumarate. No significant effect of 1 m*M* oleate on the secretory response could be detected at either high or low glucose concentration.

Table III summarizes the insulin secretory responses observed in the 90-min incubation experiments. Again, no significant effect of medium- or long-chain fatty acid was observed, at either high or low glucose concentrations.

Table IV gives data on the insulin secretory responses in 90-min experiments in which the medium did not include pyruvate, glutamate, and fumarate. Both 3 m*M* oc-

tanoate and 2 m*M* oleate were tested at high and low glucose concentrations. No significant differences in secretory response could be detected.

Discussion. Studies of the effects of free fatty acids on the release of insulin have yielded inconsistent results. Large intravenous doses of long-chain fatty acids (12), or intravenous infusions of a triglyceride emulsion plus heparin (2) have been shown to stimulate insulin secretion in dogs. Since direct infusion of FFA in man is not feasible, their effect has been studied by giving oral or intravenous fat accompanied by heparin injection. In general (13–16), little effect on basal insulin levels was noted, although the glucose-induced insulin release was enhanced. Recent experiments in which the FFA in human volunteers was raised to 3 m*M*, showed a modest rise (8 μU/ml) of circulating insulin and an enhanced insulin response to arginine. A similar study in baboons (18) showed no insulin response to FFA.

In smaller animals no *in vivo* studies have been done. The data obtained *in vitro* are also inconsistent. Sanbar and Martin (9) reported a stimulatory effect of 3 m*M* octanoate in rat pancreas slices, as did R. Candela and Salinas (19). Lambert, Jeanrenaud, Junod, and Renold (20) showed that 11 m*M* octanoate (a very high level) in the presence of 10 m*M* caffeine enhanced insulin release by cultured explants of fetal rat pancreas. Studies in isolated islets of adult rats have also exhibited octanoate-induced insulin release (21). However, octanoate had no stimulatory effect on pieces of rabbit (7) or rat (6) pancreas.

The *in vitro* effects on long-chain fatty acid are also uncertain. Thus, palmitate did not stimulate either rabbit (7) or rat (22) pancreas, while Malaisse and Malaisse-Lagae (6), although unable to demonstrate an insulin-secretory effect of palmitate (0.5 m*M*) at a glucose concentration of 3.0 mg/ml, obtained such an effect in the absence of glucose or at glucose concentrations of 1.0–1.5 mg/ml. These investigators suggested that the glucose at high concentration had maximally increased the insulin secretory rate so that an additional stimulus could have no further effect.

In the present experiments, no evidence was found for enhanced secretion of insulin by the pancreas at physiologically elevated levels (1–3 m*M*) of either medium- or long-chain FFA. This was true at either low (basal) or high glucose concentration. The results thus provide no support for the hypothesis that FFA are important insulin secretagogues.

Summary. The effect of fatty acids on insulin secretion *in vitro* was investigated. Pieces of pancreas from fed rats were incubated for 15 or 90 min at low (0.6 mg/ml) and high (3.0 mg/ml) glucose concentrations with and without either sodium octanoate or sodium oleate. A highly significant difference in insulin secretion between low and high glucose concentrations indicated viability and responsiveness of the incubated tissue. No statistically significant effect of octanoate or oleate on insulin secretion was found at either low or high glucose concentration. Thus, no support was found for the concept that medium- and long-chain free fatty acids are insulin secretagogues.

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