Increased Levels of Free Fatty Acids in Fasted Mice Stimulate In Vivo β-Cell Electrical Activity

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The electrical activity of pancreatic β-cells in 48-h fasted mice has been recorded in vivo. Their electrical activity is exceedingly high at low levels of blood glucose when compared with control animals. For example, at a blood glucose concentration of 4.5 mmol/l, at which β-cells are permanently hyperpolarized in control animals, fasted animals show continuous spiking activity. In fasted animals, hyperpolarization only occurs at glycemias below 2.2 mmol/l. As in fed animals, the electrical activity in fasted mice can be decreased or suppressed by the injection of diazoxide, indicating the participation of K_{ATP} channels. The treatment of fasted animals with nicotinic acid, an inhibitor of lipolysis, produces a decrease in the levels of free fatty acids (FFAs) and a decrease in electrical activity, thereby restoring the dose-response curve for glucose in fasted animals to values close to those found in fed animals. Conversely, the injection of palmitic acid produces an increase in electrical activity without a change in blood glucose. These results point to FFAs as important regulators of electrical activity during fasting in vivo. They also indicate a dissociation of electrical activity and insulin release in fasted animals, since an increase in electrical activity is not associated with increased insulin secretion. ***Diabetes*** **47:1707–1712, 1998**

The secretion of insulin by pancreatic β-cells is regulated by a variety of factors, among which the most important is glucose. It is accepted that metabolism of the sugar is a necessary step in the chain of events that leads to insulin secretion (1). Glucose metabolism generates diverse coupling factors that modulate the activity of channels involved in the regulation of electrical activity and the function of proteins important for secretion. Among the former, the ATP-dependent potassium channel (K_{ATP}) is regulated by the relative levels of ATP and ADP. The closure of K_{ATP} channels, resulting from an increase in the ATP-to-ADP ratio, leads to membrane depolarization and the appearance of oscillatory electrical activity (2–4). The activation of voltage-dependent calcium channels during depolarization leads to an increase in intracellular calcium, which plays a central role in insulin secretion (5). In this scenario, changes in electrical activity are a necessary intermediate step in glucose-induced insulin secretion. In fact, studies in vitro have shown a positive correlation between glucose concentration, oscillatory electrical activity, intracellular calcium oscillations, and pulsatile insulin release (6–8).

Although there is general agreement about the basic features of the fuel hypothesis, there are important aspects that are poorly understood. For instance, the specific signal(s) that links metabolism with the closure of K_{ATP} channels and insulin secretion is not known. In addition to the levels of high energy phosphates, other signals, such as adenine nucleotides (9,10) and GTP-dependent mechanisms (11), have been implicated as important regulatory factors.

The interaction between lipid and glucose metabolism is well known; however, the effects of lipid metabolism on insulin secretion are poorly understood (12). Acyl-CoA esters have been implicated in several aspects of insulin secretion. For example, they have been found to modulate K_{ATP} channels (13) and to modify intracellular calcium handling through an effect in a nonmitochondrial compartment (14). Under normal metabolic conditions, with low levels of free fatty acids (FFAs), glucose metabolism leads to a change in the intracellular lipid profile, consisting of an increase in malonyl-CoA and the suppression of fatty acid oxidation (15–18), which seems to be a key event in glucose-induced insulin secretion. During starvation, the acute phase of insulin secretion in response to glucose is impaired when measured in isolated pancreatic islets (19,20). However, in the whole animal, such a response is not greatly depressed. Recent evidence suggests that elevated levels of FFAs play an important role in the ability of islets to secrete insulin in response to glucose in starved animals (21,22). Thus, the blockade of lipolisis during starvation greatly impairs glucose-induced insulin secretion. Conversely, the exposure of islets to fatty acids results in a shift to the left of the glucose-induced insulin secretion (23). From studies in vitro and in vivo, the effect of fatty acids on β-cell function seems to be dual. Short-term exposure to fatty acids produces a stimulation of glucose-stimulated insulin secretion (16,22,24,25), whereas long-term exposure produces an impairment (26–28). This long-term inhibition of insulin secretion may be related to early forms of NIDDM, where high glycemia and high lipidemia produce overstimulation and, finally, β-cell dysfunction (29–31). The overall emergent picture is that lipids may play an important role in β-cell physiology, probably at different steps along the stimulus secretion coupling chain.

The electrical activity of pancreatic β-cells has been recently recorded in vivo (32–34). Such studies have the

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Received for publication 8 April 1998 and accepted in revised form 31 July 1998.

EC_{50}, glycemia at which the cells are in the active phase half of the time; FFA, free fatty acid.

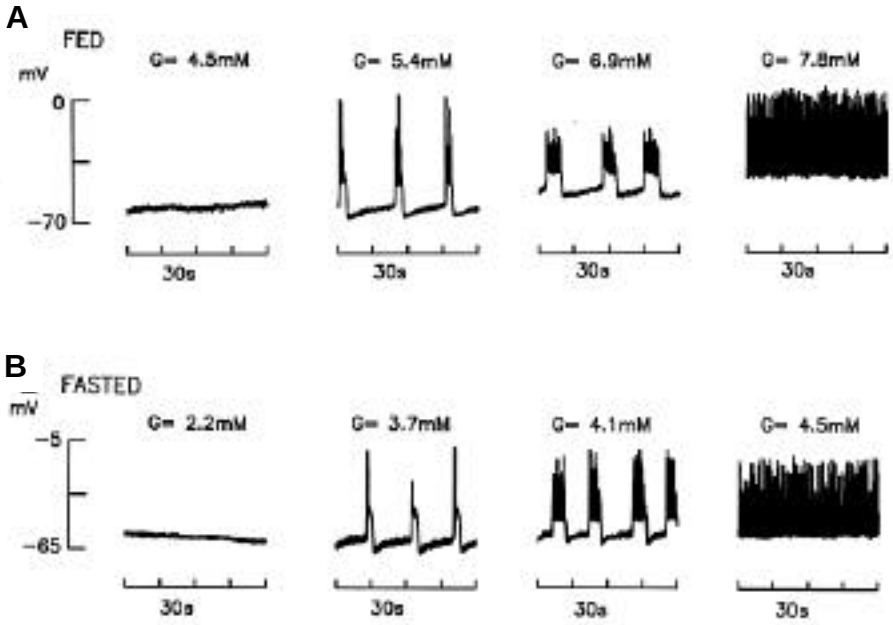


FIG. 1. Representative records of the electrical activity recorded in vivo in fed (*A*, label control) and 48-h fasted animals (*B*). The values above each record, denoted by the letter G, are the blood glucose concentrations measured during electrical recording. Each record is from a different animal.

dvantage of providing insight into factors other than glucose hat may be important in the modulation of β-cell activity. The ost relevant results from these studies related to the present experiments was the finding that glucose-dependent scillatory electrical activity is maximally sensitive to hanges in blood glucose concentration within the normal ange of glycemia, ~5–8 mmol/l. The half-maximal sensitivity n isolated islets of Langerhans is obtained at 12–15 mmol/l lucose concentration.

The present study was undertaken to investigate the relaonship between blood glucose concentration and electrical ctivity recorded in vivo under restricted metabolic condions. It was expected that in starved animals, which have a ower blood glucose concentration, electrical activity would e proportionally decreased. Contrary to our expectations, we ave found that in starved animals there is an overstimulation f electrical activity that is not associated with an increase in nsulin secretion. Such overactivity is directly related to the ncreased levels of FFAs secondary to the fasting state.

ESEARCH DESIGN AND METHODS

he methods used for in vivo recording have been described elsewhere (32). lbino mice (8–10 weeks old, 25–35 g wt) were anesthetized by intraperitoneal jection of 90 mg/kg Nembutal. Control animals had free access to food and water, hile starved animals were fasted for 48 h. The degree of anesthesia was checked eriodically during the experiment by testing cutaneous reflexes. The experiments ere carried out according to institutional animal care guidelines. The animals ere laparotomized, and the duodenal part of the pancreas was dissected free from dherence. The vena cava and the abdominal aorta were cannulated in their caual parts for solution infusion and blood sample collection, respectively. During e experiment, the animal was laid on its back on a heated bed maintained at 37°C. or the electrical recording, the duodenal part of the pancreas was spread out on p of a platform (6 × 15 mm) covered by a 3-mm layer of Sylgard (Dow Corng, Midland, MI). The platform was attached to a micromanipulator. The pancreas as isolated from peristaltic and respiratory movements by fixing it to the Sylgard ith dissection pins, taking care not to affect the blood supply. The islets were npaled with high-resistance glass microelectrodes (100–150 MΩ) filled with a soluon of 3 mol/l potassium citrate plus 100 mmol/l potassium chloride, and the electical activity was recorded with an Axoclamp-2A amplifier (Axon Instruments, oster City, CA). Unfiltered records were acquired at a frequency of 300 Hz and ored on a microcomputer using Axotape for analysis off-line. Blood samples were ollected from the aorta and analyzed for glucose concentration (Beckman glose analyzer 2; Beckman, Brea, CA), insulin concentration (Coat-A-Count; Diagstic Products, Los Angeles, CA) and nonesterified fatty acids (FFAs) (half-icro test; Boehringer Mannheim, Mannheim, Germany).

For in vitro experiments, islets were isolated from the pancreas by dissection and fixed to the base of a recording chamber. Islets were superfused with a modified Krebs solution containing (in millimoles per liter) 120 NaCl, 5 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, and 1.1 $MgCl_2$ and gassed with a mixture of 95% O_2 and 5% CO_2 (pH = 7.4). These experiments were carried out at 37ºC with different glucose concentrations. All the reagents were of analytical grade from Sigma (Poole, U.K.) and Merck (Darmstart, Germany).

RESULTS

Figure 1 shows examples of the electrical activity recorded in vivo from fed (Fig. 1*A*) and 48-h fasted (Fig. 1*B*) animals. The numbers above each record correspond to the glycemia measured simultaneously with the electrical recording, which is representative of electrical activity under steady-state conditions. In fed animals, at a blood glucose of 4.5 mmol/l the cell is below threshold and its membrane potential is hyperpolarized. At 5.4 mmol/l glucose, the cell shows oscillations in the electrical activity composed of alternating depolarized and hyperpolarized phases. At a higher blood glucose concentration (6.9 mmol/l), active phases of longer duration and shorter silent phases are seen. Finally, when blood glucose is 7.8 mmol/l, the cell displays continuous activity. This relationship between blood glucose and electrical activity is consistently found in in vivo electrical recordings (32).

Figure 1*B* shows that for a given glycemia, the activity is consistently higher in fasted animals compared with the control ones. For example, 4.5 mmol/l blood glucose, a concentration that is subthreshold in control animals, in fasted animals produces maximal activation of the cell. In fasted animals with lower glucose concentrations, 4.1 and 3.7 mmol/l, the electrical activity is progressively decreased. Only glycemias as low as 2.2 mmol/l are associated with cell hyperpolarization.

Figure 2 shows the relationship between blood glucose and the percentage of active phase (the time spent depolarized with respect to the total recording time) for fasted animals. In this figure, each open circle corresponds to a fasted animal recording (n = 67), such as those shown in Fig. 1*B*. The data points indicate a very steep relationship between blood glucose and electrical activity. The cells become active at glycemias in the range 2.5–3 mmol/l and show continuous

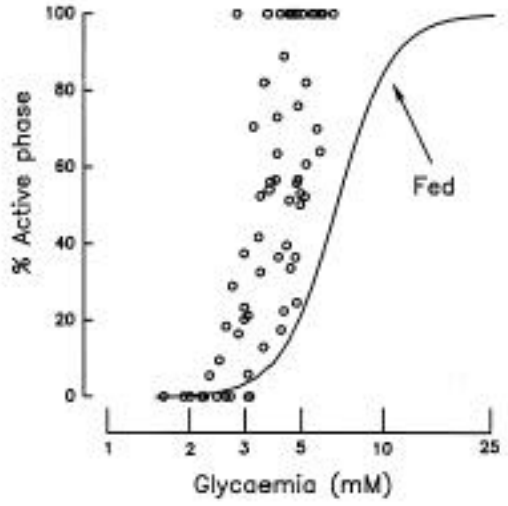


FIG. 2. Plot of the relationship between glycemia and percentage of the active phase of electrical activity. Each open symbol represents a different experiment in fasted animals (n = 67), like those shown in Fig. 1*B*. The sigmoid curve, labeled "Fed," shows the fitting obtained in experiments on fed animals, like those shown in Fig. 1*A* (32). The EC_{50} (glycemia at which the cells are in the active phase half of the time) for the control curve is 6.76 mmol/l (95% CI 6.59–6.94), and the slope is 5.57 (95% CI 4.67–6.50). A sigmoidal fitting to the data obtained in fasted animals gives an EC_{50} of 3.8 mmol/l (95% CI 3.5–4.1) and a slope of 4.6 (95% CI 2.9–6.2).

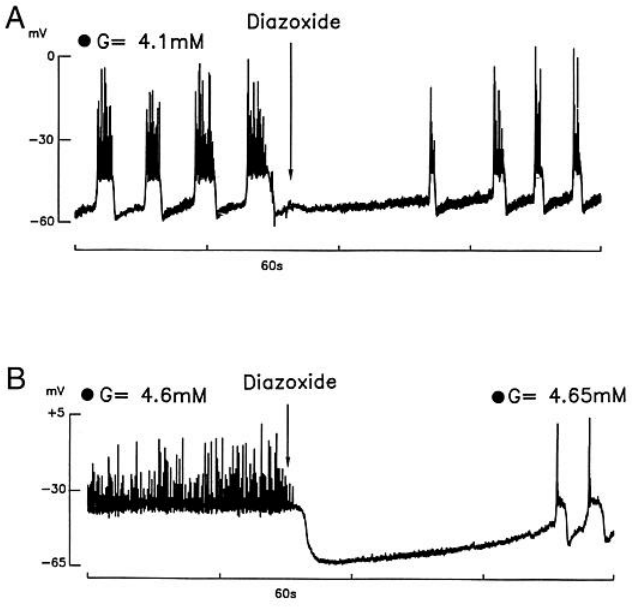


FIG. 3. Effect of diazoxide on the electrical activity in fasted animals. In *A*, diazoxide was injected (50 µl of a 15 mmol/l solution) at the time indicated by the arrow into an animal that shows oscillatory electrical activity. *B* shows the effect of the same diazoxide concentration in an animal with continuous electrical activity. The letter G denotes the time at which a blood sample was taken and the glycemia measured. The figure is representative of six similar experiments.

electrical activity above 5 mmol/l. In other words, in the narrow range from 2.5 to 5 mmol/l glucose, which are the values of glycemias in our fasted animals, the cells change from no activity to maximal activity. The continuous line labeled "Fed" shows the fitting of data obtained in previous experiments (32) and describes the relationship between blood glucose and electrical activity in fed animals, such as those shown in Fig. 1*A*. It is clear that in fasted animals, for a given blood glucose concentration, the cells are more active than those in the control situation. Thus, contrary to the expected results that β-cell electrical activity would decrease with the lower glycemias associated with fasting, we observed hyperactivity and a shift of the dose-response curve toward lower glucose levels.

One possible explanation for the hyperactivity observed in fasted animals could be the impairment of cell metabolism due to hypoglycemia. We considered the possibility that the K_{ATP} channels were blocked and unresponsive. To test this possibility, we looked at the effects of the K_{ATP} channel opener diazoxide on electrical activity in fasted animals.

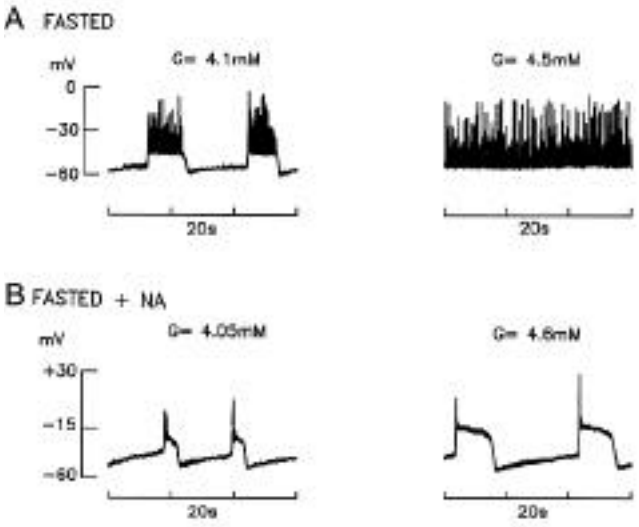
Figure 3*A* shows the effect of diazoxide on electrical activity in a fasted animal with a glycemia of 4.1 mmol/l. The injection of diazoxide leads to the transitory suppression of the active phases for a period of ~90 s, after which the cell reestablished the oscillatory pattern. Figure *3B* shows the effect of diazoxide on a different cell, which showed continuous electrical activity. Again, diazoxide completely hyperpolarized the cell, in this case for a longer period of time. The effect was transitory, and the cell restarted electrical activity, in this case with oscillations of electrical activity. Essentially the same diazoxide modulatory effects have been described in control animals (35). These results suggest, therefore, that in fasted animals, the electrical activity of pancreatic β-cells is controlled by the K_{ATP} channel activity, although with a different glucose sensitivity.

The shift in the dose-response curve for glucose found in fasted animals could be due to an intrinsic change in the β-cell sensitivity to glucose. Alternatively, the factor responsible for the shift could be external to the β-cells. If the former is correct, isolated islets from fasted animals should show a different dose-response curve for glucose with respect to islets taken from control animals. To check this, we isolated islets from control and fasted animals and recorded the electrical activity at different glucose concentrations. The time taken from the isolation of the islets and the beginning of the recording ranged from 30 to 60 min. We found in both groups of islets the same dose-response relationship (not shown). These results suggested that there may be a factor in fasted animals whose presence changes β-cell glucose sensitivity.

TABLE 1
Glycemia, insulin, and FFAs in the different groups of animals studied

	Glycemia (mmol/l)	Insulin (µU/ml)	FFAs (mmol/l)
Control	7 ± 1.2 (73)	10.3 ± 3.5 (5)	0.2 ± 0.05 (11)
Fasted	4.7 ± 0.8 (64)*	5.9 ± 0.8 (7)†	0.65 ± 0.37 (11)*
Control + NA	7.4 ± 1.7 (8)	9.5 ± 2.8 (5)	0.12 ± 0.05 (5)
Fasted + NA	4.4 ± 0.6 (13)*	7.4 ± 2.5 (7)	0.15 ± 0.07 (9)

Data are means ± SD (n). Groups of animals studied include control (fed), 48-h fasted, control treated with nicotinic acid (NA), and fasted treated with NA. *P < 0.01; †P < 0.05 (fasted compared with control and fasted + NA compared with control + NA).



IG. 4. Representative records of the electrical activity recorded in 48-fasted animals (*A*) and fasted animals treated with nicotinic acid (*B*). he figure is representative of the effects obtained in 12 animals eated with nicotinic acid. The values above each record, denoted by ıe letter G, are the blood glucose concentrations measured during lectrical recording. Each record is from a different animal.

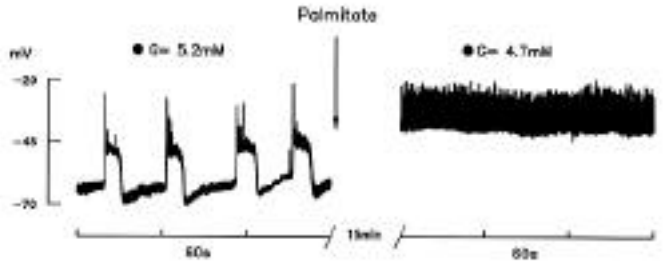


FIG. 5. Effect of the injection of palmitate (25 µl of a 5 mmol/l solution) on the electrical activity in fasted animals treated with nicotinic acid. Palmitate was injected at the end of the first part of the record, at the time indicated by the arrow. The second part of the record shows the electrical activity 15 min after the injection of palmitate. The letter G denotes the time at which a blood sample was taken and the glycemia measured. The figure is representative of six similar experiments.

The most notable change produced during fasting is an ıcrease in circulating FFAs. As shown in Table 1, under our xperimental conditions the FFA values rose from 0.2 mmol/l ı control animals to 0.65 mmol/l in fasted animals. The treatıent of fasted animals with the antilipolytic agent nicotinic cid (36) prevented the rise in FFAs during fasting, whereas had no effect in control animals (Table 1).

Figure 4 shows the effect of treatment with nicotinic acid ı fasted animals. The nicotinic acid treatment consists of aily intraperitoneal injections of 30 µl (stock 100 mmol/l) of icotinic acid. Figure 4*A* shows the electrical activity at two lucose concentrations (4.1 and 4.5 mmol/l) in fasted aniıals. As previously shown (Fig. 1), cells at these glycemias ange from moderately activate at 4.1 mmol/l to continuously ctive at 4.5 mmol/l glucose. Figure 4*B* shows electrical activy in two animals treated with nicotinic acid. As can be ppreciated, for comparable blood glucose concentrations the lectrical activity is considerably lower in these animals and ; very close to the activity recorded in control nonfasted aniıals. In fact, the percentage of active phase for a given glyemia in fasted animals treated with nicotinic acid is the ame as the one obtained in control animals (represented in ig. 2 by the continuous line).

In parallel experiments, we checked the effect of nicotinic cid in normally fed animals. As shown in Table 1, nicotinic cid treatment did not produce any significant change in lood glucose or insulin secretion and produced only a small ecrease in FFAs. The electrical activity recorded from fed aniıals treated with nicotinic acid was indistinguishable from ıat recorded from fed nontreated animals (data not shown).

If the FFA levels are responsible for increased electrical ctivity in fasted animals, it should be possible to mimic the asted state by the addition of fatty acids. Figure 5 shows the ffect of palmitic acid injection on electrical activity in a asted and nicotinic acid–treated animal. The initial part of the record shows the electrical activity, which is equivalent to that of a fed animal, since lipolysis was prevented by nicotinic acid. At the time indicated by the arrow, palmitate was injected, leading to a slow increase in electrical activity. At 15 min after the injection, the cell was in continuous electrical activity, although the glucose concentration slightly decreased. In six experiments performed under similar conditions, palmitate produced an increase in the length of the active phase of electrical activity of 111 ± 94% (mean ± SD). The glycemia values before and after injection of palmitate were 4.9 ± 0.5 and 4.8 ± 0.5 mmol/l, respectively.

DISCUSSION

The results presented in this paper show that for a given blood glucose concentration, electrical activity of pancreatic β-cells in fasted animals is increased with respect to control animals. This increase in activity seems to be produced by the increase in FFAs that occurs during fasting. These results put forward two important points. First, lipid metabolism exerts an important influence on pancreatic β-cell electrical activity. Second, in fasting animals, there is a dissociation between electrical activity and insulin secretion. Here, increased electrical activity is not associated with an increase in insulin secretion.

Fasting leads to a decrease in blood glucose levels and a corresponding decrease in circulating insulin. Taking into consideration previous electrophysiological recordings in vivo (32,35), a proportional decrease in electrical activity was expected; however, the opposite effect was found. For a given blood glucose concentration, the electrical activity is higher in fasted animals. The overstimulated electrical activity observed in 48-h fasted animals with respect to fed ones is due to a specific metabolic effect, not to impairment of β-cell status or gross alterations in the channels implicated in regulation of membrane potential. As in fed animals, the membrane potential of β-cells in fasted animals can be regulated by pharmacological interventions that modulate K_{ATP} channels. Thus, treatment with the K_{ATP} channel opener diazoxide led to hyperpolarization of the membrane and a decrease in the time spent by the cell in the active phase. Likewise, the K_{ATP} channel blocker tolbutamide increases electrical activity in hyperpolarized cells (data not shown). The same pharmacological modulation has been reported in fed animals (35). On the other hand, islets of Langerhans dissected

from fasted animals show the same glucose–electrical activity relationship as fed ones.

The increased electrical activity in fasted animals is a consequence of increased levels of FFAs. This conclusion is derived from the experiments with nicotinic acid and the effects of palmitic acid infusion. Thus, the effect of fasting on electrical activity can be blocked by treatment with the antilipolytic agent nicotinic acid (36), restoring the relationship between glucose and electrical activity toward the fed type. Conversely, infusion of palmitic acid leads to the apparition of increased electrical activity. The slow time course of the palmitic acid effect may indicate that it is not due to a direct effect on membrane channels, but to an indirect one dependent on metabolism.

It has been recently shown that long-chain acyl-CoA esters produce the activation of K_{ATP} channels (13). Such an effect should lead to a hyperpolarization rather than the increased electrical activity observed here. It is possible that differences in experimental conditions may explain such differences. It is also possible that the effects of lipids on K_{ATP} channels are different in short- and long-term exposures (see below). Further work is necessary to clarify this point.

There is a body of work suggesting the importance of lipid metabolism in β-cell insulin secretion. The effects of lipids have been implicated in the normal physiological response to glucose, as well as that under pathological conditions. Under physiological conditions, glucose metabolism leads to the diversion of fatty acids from the oxidative to the sterification pathway, leading to an increase in fatty acyl-CoA (15–18). This diversion is due to an increase in malonyl-CoA and its inhibitory action on carnitine palmitoyl-transferase I (CPTI). However, the exact mechanism(s) that links the increase in FA-CoA with insulin secretion is not clear and could be direct or mediated by increased pools of diacylglycerol or the effect on other enzymes proximal to insulin secretion. There is also evidence that the effect of fatty acids on insulin secretion depends on the length of exposure. Short-term exposure produces a stimulation (16,22,24,25), whereas long-term exposure produces an inhibition of insulin secretion (26–28). In this respect, the increased lipid levels in 48-h fasting mice has to be considered long-term exposure. Consistent with this, the fasted animals treated with nicotinic acid show a tendency to increase insulin levels (5.9 vs. 7.4 μU/ml, fasted vs. fasted plus nicotinic acid) despite the fact that the values of glycemia still correspond to those of fasted animals (4.7 vs. 4.4 mmol/l, fasted versus fasted plus nicotinic acid). It has to be kept in mind that the increased insulin secretion in nicotinic acid–treated animals is associated with a decrease in the electrical activity. This further highlights the dissociation between electrical activity and insulin secretion in fasted animals.

The existence of a positive correlation among glucose concentration, electrical activity, and the concomitant increase in intracellular calcium and insulin secretion in models in vitro and in vivo is well documented. This correlation seems to be modified during starvation. Our experiments do not provide information to clarify the steps that may be altered. However, it is interesting to note that in isolated HIT cells, acyl-CoA esters cause an increase in calcium influx into a nonmitochondrial pool, consistent with a stimulatory effect on the endoplasmic reticulum Ca-ATPase. Therefore, it would be interesting to determine if overstimulation of electrical activity in fasted animals is associated with the expected increased levels of intracellular calcium (37). In the case of a prevailing effect on the Ca-ATPase, the calcium increase due to electrical activity could be at least partially curtailed. In addition, it has been shown that fasted rats show reduced levels of mRNA for the calcium channel (38). Further experiments are necessary to check if the increased electrical activity is associated with increased levels of intracellular calcium.

Previous work has shown that during fasting, the presence of FFAs is necessary for an adequate insulin secretory response to an increase in glucose (21). Treatment with nicotinic acid leads to a decrease in such a response. From our experiments, it can be speculated that in nicotinic acid–treated animals, which have a fed-like glucose–electrical activity relationship, the secretory response to a glucose load would be decreased. This may indicate that in addition to electrical activity, there must be other factors directly implicated in insulin secretion. In any case, the final effects of FFAs are not easily predictable because of the multiplicity of their actions.

The possible participation of elevated levels of fatty acids in initial forms of type 2 diabetes, especially those associated with obesity, has been indicated. The experiments presented here raise the possibility that continuous overstimulation of β-cell electrical activity may lead to a deleterious effect on the β-cell, contributing to the later impairment of insulin secretion.

In conclusion, our results directly demonstrate the participation of lipids in the regulation of pancreatic β-cell electrical activity during fasting. Their effect has proven to be complex and linked to a dissociation in the conventional stimulus-secretion coupling chain in pancreatic β-cells. Among other issues, it remains to be determined whether the increased electrical activity and the depressed insulin secretion are causally related or are two independent manifestations of lipid metabolism.

ACKNOWLEDGMENTS

This work was supported by grants FIS 94/1345 (Fondo de Investigaciones de la Seguridad Social) and SAF 97/0195 (Comision Interministerial de Ciencia years Tecnologia, Spain).

We thank A. Perez-Vergara for technical assistance.

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Author Queries (please see Q in margin and underlined text)

Q1: Is this the correct affiliation for both authors? If not, please provide affiliations.
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Q7: Correct that you are referring here to the sigmoid curve labeled “Fed” in Fig 2? “fed” OK for “control” here?
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References
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