Biochemical Pharmacology, Vol. 55, pp. 909–913, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00531-5

SHORT COMMUNICATION

Stimulation of Biosynthetic Activity by Novel Succinate Esters in Rat Pancreatic Islets

Aouatif Laghmich, Laurence Ladrière,* Francine Malaisse-Lagae,* Heinz Dannacher,† Fredrik Björkling† and Willy J. Malaisse*‡*

*Laboratory of Experimental Medicine, Brussels Free University, Brussels, Belgium
†Leo Pharmaceutical Products, Ballerup, Denmark

ABSTRACT. Selected esters of succinic acid are currently under investigation as possible insulinotropic agents for the treatment of noninsulin-dependent diabetes mellitus. The aim of the present study was to investigate the effects of ten novel esters of succinic acid upon biosynthetic activity in rat pancreatic islets. In the absence of any other exogenous nutrient, glycerol-3-hydroxy-1,2-dimethyl succinate (0.5 mM), D-arabitol-5-hydroxy-1,2,3,4-tetramethylsuccinate (0.5 mM), and 4-tert-butylsuccinate (2.5 mM) exerted little or no effect upon L-[4-^{3}H]phenylalanine incorporation into trichloroacetic acid-precipitable material. A modest but significant increase in biosynthetic activity to approximately 150% of basal value was found in the presence of L-threitol-1,2,4-trimethylsuccinate (2.0 mM) and ethanediol-1,2-diethylsuccinate (2.5 mM). A two- to five-fold increase in protein biosynthesis was observed in islets exposed to propanediol-1,2-dimethylsuccinate, glycerol-1,2-dimethylsuccinate-3-hydrogenosuccinate, L-threitol-3-succinoyl-1,2,4-trimethylsuccinate, glycerol-1,2-dimethylsuccinate or ethanediol-1,2-dimethylsuccinate (2.5 mM each), these esters being mentioned in order of increasing biological efficiency. There was a significant correlation between these results and the insulinotropic action of the same esters. The present findings thus reinforce the view that such esters act as nutrients in islet cells and, therefore, offer the advantage over pharmacological agents currently used for the treatment of type-2 diabetes in stimulating both the biosynthetic and secretory activity of insulin-producing B-cells. BIOCHEM PHARMACOL **55**;6:909–913, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. rat pancreatic islets; protein biosynthesis; succinate esters

Esters of succinic acid are currently under consideration as potential nutrients in cells endangered by an imbalance between ATP synthesis and breakdown. For instance, they were found to prevent or delay the metabolic and hormonal consequences of starvation [1–4]. They may also protect against mortality caused by endotoxemia [5]. Moreover, they were proposed as insulinotropic agents in the treatment of noninsulin-dependent diabetes mellitus [6]. In the latter perspective, the esters first investigated, such as the monomethyl and dimethyl esters of succinic acid, presented the disadvantage of only exerting a sizeable stimulation of insulin release either when tested *in vitro* at high concentrations or administered in relatively large amounts *in vivo* [7, 8]. Recently, however, novel esters of succinic acid have been developed that stimulate insulin release when either used *in vitro* in the micromolar range or given *in vivo* in amounts not exceeding 0.07 μmol/g body weight [9, 10]. The major aim of the present study was to explore whether these novel esters also offer the advantage of stimulating biosynthetic activity in rat pancreatic islets.

MATERIALS AND METHODS

L-[4-^{3}H]phenylalanine (27–29 Ci/mmol) was purchased from Amersham International. The ten esters examined in the study (Fig. 1) were 4-tert-butyl-succinate (TB-1S)§, ethanediol-1,2-dimethylsuccinate (G-2MS), ethanediol-1,2-diethylsuccinate (G-2ES), propanediol-1,2-dimethylsuccinate (P_2-2MS), glycerol-1,2-dimethylsuccinate (Gl-2MS), glycerol-3-hydroxy-1,2-dimethylsuccinate (P_4-2MS), glycerol-1,2-dimethylsuccinate-3-hydrogenosuccinate (Gl-2MS,S), L-threitol-1,2,4-trimethylsuccinate (Th-3MS), D-arabitol-5-hydroxy-1,2,3,4-tetramethylsuccinate (Pe-4MS), and L-threitol-3-succinoyl-1,2,4-trimethylsuccinate (Th-3MS,S). They are ranked in this enumeration according to the number of succinyl residues (S) in each molecule. They were prepared by direct esterification of the corresponding polyol by the

‡ Corresponding author: Prof. W. J. Malaisse, Laboratory of Experimental Medicine, Brussels Free University, 808 Route de Lennik, B-1070 Brussels, Belgium. TEL: (32) 2 555 62 37; FAX: (32) 2 555 62 39.
Received 17 June 1997; accepted 10 September 1997.

§ *Abbreviations:* Gl-2MS, glycerol-1,2-dimethylsuccinate; Gl-2MS,S, glycerol-1,2-dimethylsuccinate-3-hydrogenosuccinate; G-2ES, ethanediol-1,2-diethylsuccinate; G-2MS, ethanediol-1,2-dimethylsuccinate; Pe-4MS, D-arabitol-5-hydroxy-1,2,3,4-tetramethylsuccinate; P_4-2MS, glycerol-3-hydroxy-1,2-dimethylsuccinate; P_2-2MS, propanediol-1,2-dimethylsuccinate; TB-1S, 4-tert-butyl-succinate; TCA, trichloroacetic acid; Th-3MS, L-threitol-1,2,4-trimethylsuccinate; Th-3MS,S, L-threitol-3-succinoyl-1,2,4-trimethylsuccinate.

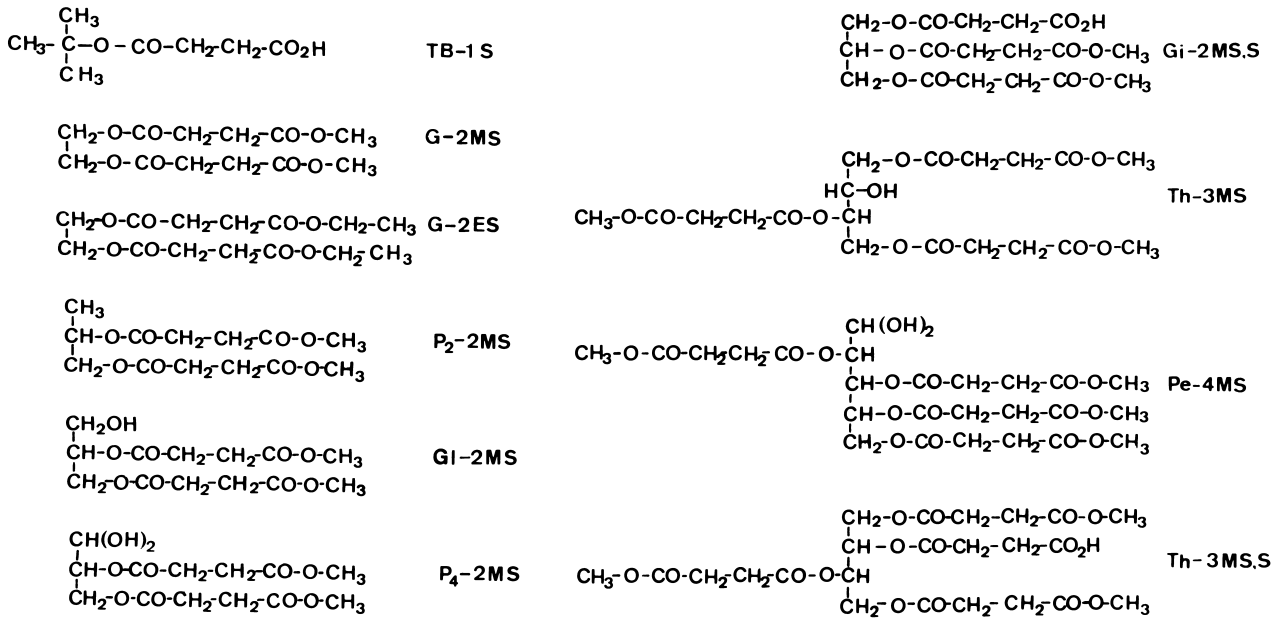

FIG. 1. Chemical structure of the ten esters examined in this report.

desired succinic acid derivative using previously reported procedures (Patent application UK 9612331.0, 1996).

All experiments were conducted in pancreatic islets isolated by the collagenase technique [11] from fed female Wistar rat (180–220 g, Proefdierencentrum), and incubated in groups of 20 islets each over 90 min at 37° in 50 μL of a bicarbonate-buffered medium [11] containing bovine serum albumin (5 mg/mL) and L-[4-^{3}H]phenylalanine (3.5 μM). The esters of succinic acid were incorporated into the incubation medium from stock solutions prepared in DMSO. The same concentration of the solvent (1%, v/v) was present in control (no ester) and test media. At this concentration, DMSO fails to affect islet function [12].

After incubation, the islets were rinsed twice with 1.0 mL of the same bicarbonate-buffered medium now containing 1.0 mM unlabelled L-phenylalanine and sonicated on ice (10 sec at 10 μm, Soniprep 150; MSE) in 0.8 mL of acetic acid (2.0 M). Two aliquot portions (25 μL each) of each homogenate were examined by liquid scintillation to measure the total radioactive content of the islets. Two further aliquots (50 μL each) were mixed with 0.45 mL of a glycine-NaOH buffer (0.2 M, pH 8.8) and 0.5 mL of TCA (20%, v/v). After centrifugation for 5 min at 1,000 *g*, 0.5 mL of the supernatant was also examined for its radioactive content (TCA-soluble material). The amount of tritiated TCA-precipitable materiel could be determined by calculating the difference between these two sets of measurements. After correction for the blank values obtained under identical conditions in media that had been incubated in the absence of islets, the results were expressed by reference to the specific radioactivity of L-[4-^{3}H]phenylalanine in the incubation medium.

All results are expressed as mean values (±SEM), together with the number of individual determinations (N). The statistical significance of differences between mean values was assessed by use of Student's t-test.

RESULTS

In the present series of experiments, the incorporation of L-[4-^{3}H]phenylalanine (3.5 μM) into TCA-precipitable material averaged, in islets exposed to 16.7 mM glucose, 704.8 ± 16.6% (n = 105) of the mean basal value (100.0 ± 4.4%; n = 102) found within the same experiments in islets deprived of exogenous nutrient (Table 1). The pool of TCA-soluble tritiated material was also higher ($P < 0.005$) in glucose-stimulated islets (14.7 ± 1.1 fmol/islet) than in glucose-deprived islets (10.6 ± 0.3 fmol/islet). Nevertheless, the paired ratio between TCA-precipitable and total islet radioactive content was higher ($P < 0.001$) in glucose-stimulated islets (97.5 ± 0.1%) than in nutrient-deprived islets (88.3 ± 1.1%).

In the absence of exogenous nutrient, TB-1S (2.5 mM) and P_4-2MS (0.5 mM) failed to affect the *de novo* biosynthesis of tritiated islet peptides. A minor increase in biosynthetic activity to 132.2 ± 16.8% (n = 11) of mean basal value was observed in islets exposed to Pe-4MS (0.5 mM), an effect only achieving statistical significance ($P < 0.05$) in one out of two sets of comparisons made within the same experiment. A somewhat greater stimulation of protein biosynthesis was found in islets exposed to either G-2ES (2.5 mM) or Th-3MS (2.0 mM), the measurements averaging, respectively, 141.5 ± 10.5% (n = 11; $P < 0.02$) and 147.5 ± 19.3% (n = 11; $P < 0.05$) of the mean corresponding basal value.

All other esters, which were always tested at a 2.5 mM concentration, increased the incorporation of L-[4-^{3}H]phe-

TABLE 1. Effect of succinic acid esters upon the incorporation of L-[4-^{3}H]phenylalanine into TCA-precipitable and TCA-soluble material

D-glucose* (mM)	Ester (mM)	TCA-precipitable (fmol/islet per 90 min)	TCA-soluble (fmol/islet per 90 min)	TCA-precipitable (% of total)
		77.3 ± 5.2 (12)	10.8 ± 0.6 (12)	87.6 ± 1.1 (12)
	TB-1S (2.5)	83.8 ± 8.2 (11)	9.4 ± 1.1 (11)	88.5 ± 1.8 (11)
16.7		612.0 ± 33.5 (12)	17.1 ± 2.4 (12)	97.3 ± 0.3 (12)
		95.6 ± 10.1 (11)	13.0 ± 3.8 (11)	88.9 ± 1.6 (11)
	G-2MS (2.5)	435.2 ± 39.7 (11)	13.2 ± 2.7 (11)	96.7 ± 0.4 (11)
16.7		714.8 ± 53.9 (11)	14.6 ± 2.9 (11)	97.6 ± 0.5 (11)
		65.0 ± 6.8 (11)	4.7 ± 1.7 (11)	90.1 ± 1.4 (11)
	G-2ES (2.5)	92.0 ± 6.8 (11)	4.1 ± 1.0 (11)	93.1 ± 0.6 (11)
16.7		458.1 ± 62.1 (12)	6.9 ± 1.2 (12)	97.9 ± 0.2 (12)
		109.6 ± 10.4 (11)	13.8 ± 1.9 (11)	91.1 ± 2.2 (11)
	P_2-2MS (2.5)	230.5 ± 25.0 (10)	11.6 ± 1.1 (10)	97.7 ± 0.3 (10)
16.7		803.1 ± 59.9 (12)	16.0 ± 1.0 (12)	98.2 ± 0.2 (12)
		80.2 ± 6.6 (12)	8.8 ± 1.9 (12)	88.4 ± 7.3 (12)
	Gl-2MS (2.5)	249.4 ± 28.3 (12)	13.2 ± 3.2 (12)	94.2 ± 0.7 (12)
16.7		495.8 ± 21.4 (12)	15.3 ± 2.0 (12)	96.4 ± 0.4 (12)
		54.5 ± 10.0 (11)	5.1 ± 1.0 (11)	89.7 ± 2.0 (11)
	P_4-2MS (0.5)	60.2 ± 3.8 (11)	5.7 ± 0.9 (11)	91.2 ± 1.5 (11)
16.7		421.6 ± 31.6 (11)	9.1 ± 1.3 (11)	97.9 ± 0.3 (11)
		94.2 ± 16.7 (12)	23.1 ± 3.2 (12)	79.6 ± 3.1 (12)
	Gl-2MS,S (2.5)	203.9 ± 16.1 (12)	20.6 ± 2.7 (12)	90.1 ± 1.6 (12)
16.7		1054.6 ± 52.8 (12)	34.3 ± 3.7 (12)	96.8 ± 0.3 (12)
		64.9 ± 6.3 (11)	4.7 ± 1.6 (11)	90.1 ± 1.4 (11)
	Th-3MS (2.0)	95.7 ± 12.5 (11)	4.1 ± 1.0 (11)	94.8 ± 1.0 (11)
16.7		435.4 ± 21.5 (12)	5.9 ± 1.1 (12)	98.3 ± 0.3 (12)
		104.5 ± 14.4 (11)	10.5 ± 2.5 (11)	89.9 ± 2.5 (11)
	Pe-4MS (0.5)	138.1 ± 17.6 (11)	7.6 ± 1.0 (11)	94.1 ± 1.2 (11)
16.7		672.8 ± 32.0 (11)	12.4 ± 1.8 (11)	97.7 ± 0.5 (11)
		104.5 ± 14.4 (11)	10.5 ± 2.5 (11)	89.9 ± 2.5 (11)
	Th-3MS,S (2.5)	231.7 ± 16.0 (11)	8.1 ± 2.0 (11)	96.8 ± 0.5 (11)
16.7		672.8 ± 32.0 (11)	12.4 ± 1.8 (11)	97.7 ± 0.5 (11)

* In each series of experiments, the results obtained in glucose-deprived islets, whether in the absence or presence of the ester, were compared to those found in islets exposed to 16.7 mM D-glucose.

nylalanine into TCA-precipitable material to a much more marked extent, the results expressed relative to basal value ranging from 210.3 ± 22.8% in the case of P_2-2MS to 455.2 ± 41.5% in the case of G-2MS.

Figure 2 illustrates the correlation ($r = 0.664$; $P < 0.05$) between the ester-induced increment in biosynthetic activity relative to basal value and the corresponding relative increase in insulin release evoked by the same nutrient in islets exposed to 7.0 mM D-glucose [13]. In order to remain in the range of values in which the insulinotropic action of the esters is close-to-proportional to their concentration, the secretory data refer to measurements made in the 0.1 to 0.5 mM range, the ratio between the concentrations of each ester in the biosynthetic/secretory experiments being identical in all cases.

DISCUSSION

The present results unambiguously indicate that most esters under consideration here stimulate biosynthetic activity in rat pancreatic islets. It should be underlined that these esters were tested in the absence of any other exogenous nutrient. Hence, the ester concentration required to enhance protein synthesis under more physiological conditions, e.g. in the presence of D-glucose, could well be much lower than that used in the present experiments, as indeed already documented when investigating the insulinotropic action of these novel nutrients. The latter proposal is reinforced by the existence of a significant correlation between the biosynthetic and secretory efficiencies of such esters. The molecular determinants responsible for differences in biological potency between distinct esters have already been discussed elsewhere [13].

It is likely that the stimulation of islet biosynthetic activity, as documented in this study, involves an increased generation of proinsulin. In a prior study, it was observed that glycerol-1,2,3-trimethylsuccinate indeed augments the *de novo* synthesis of both proinsulin and nonhormonal peptides in islets incubated in the presence of 4.2 mM D-glucose, with a preferential effect on hormonal synthesis [10]. Since the synthesis of proinsulin accounts for half or more of total protein synthesis in nutrient-stimulated islets, a 2- to 3-fold increase in total protein synthesis, as often observed in the present study, most probably also implies enhanced biosynthesis of proinsulin.

None of the esters tested in the present work signifi-

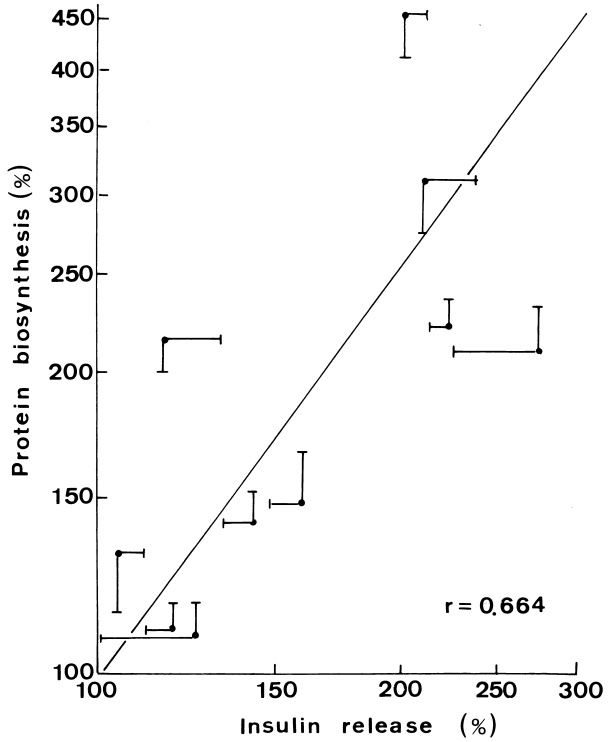

FIG. 2. Correlation between the rate of protein biosynthesis, relative to paired basal value, and the rate of insulin release, relative to paired control value, in islets exposed to ten distinct succinic acid esters. The secretory data were obtained in islets incubated in the presence of 7.0 mM D-glucose under conditions yielding increments in insulin output close-to-proportional to the ester concentration (0.1 to 0.5 mM) with, in all cases, the same ratio between such a concentration and that used in the present experiments. Mean values (±SEM) for both protein biosynthesis and insulin release are ranged on logarithmic scales.

cantly affected the size of the intracellular pool of free L-[4-^{3}H]phenylalanine, strongly suggesting that their effect on the labelling of TCA-precipitable material was not attributable to a primary effect on the net uptake of the tritiated amino acid by the islet cells.

The pharmacological drugs currently used or recently introduced as insulinotropic agents for the treatment of type-2 diabetes, e.g. hypoglycemic sulfonylureas and meglitinide analogues, fail to modify and may even adversely affect the synthesis of proinsulin and other islet peptides in pancreatic islets [14–16]. This contrasts with the ability of nutrient secretagogues to stimulate both biosynthetic and secretory activities in the insulin-producing B-cells [17–20]. The esters of succinic acid, first introduced by MacDonald and coworkers as insulin secretagogues [21–23] and now considered as possible therapeutic tools in noninsulin-dependent diabetes, offer the considerable advantage of enhancing both the generation and release of insulin.

In conclusion, therefore, the present work reinforces the view that selected esters of Krebs cycle intermediates or their precursors display favourable attributes to improve the functional behaviour of islet B-cells in type-2 diabetic patients.

This study was supported by a Concerted Research Action of the French Community of Belgium (94/99-183) and a grant from the Belgian Foundation for Scientific Medical Research (3.4513.94). The authors are grateful to C. Rémion for technical assistance and C. Demesmaeker for secretarial help.

References

1. Conget I, Zhang T-M, Eizirik DL and Malaisse WJ, SAM prevents impairment of glucose-stimulated insulin secretion caused by hexose deprivation or starvation. *Am J Physiol* **268:** E580–E587, 1995.
2. Eizirik DL, Welsh N, Sener A and Malaisse WJ, Protective action of succinic acid monomethyl ester against the impairment of glucose-stimulated insulin release caused by glucopenia or starvation: Metabolic determinants. *Biochem Med Metab Biol* **53:** 34–45, 1994.
3. Ladrière L, Zhang T-M and Malaisse WJ, Effects of succinic acid dimethyl ester infusion on metabolic, hormonal and enzymatic variables in starved rats. *J Parent Enter Nutrit* **20:** 251–256, 1996.
4. Ladrière L and Malaisse WJ, Nutritional value of succinic acid monoethyl ester in starvation. *Ann Nutr Metab* **41:** 118–125, 1997.
5. Malaisse WJ, Bakkali Nadi A, Ladrière L and Zhang T-M, Protective effects of succinic acid dimethyl ester infusion in experimental endotoxemia. *Nutrition* **13:** 330–341, 1997.
6. Malaisse WJ, The esters of carboxylic nutrients as insulinotropic tools in non-insulin-dependent diabetes mellitus. *Gen Pharmac* **26:** 1133–1141, 1995.
7. Malaisse WJ, Rasschaert J, Villanueva-Peñacarrillo ML and Valverde I, Respiratory, ionic and functional effects of succinate esters in pancreatic islets. *Am J Physiol* **264:** E428–E433, 1993.
8. Vicent D, Villanueva-Peñacarrillo ML, Malaisse-Lagae F, Leclercq-Meyer V, Valverde I and Malaisse WJ, *In vivo* stimulation of insulin release by succinic acid methyl esters. *Arch Int Pharmacodyn* **327:** 246–250, 1994.
9. Garcia-Martinez JA, Villanueva-Peñacarrillo ML, Valverde F, Björkling F and Malaisse WJ, Stimulation of insulin release and potentiation of the insulinotropic action of antidiabetic agents by 1,2,3-tri(methylsuccinyl)glycerol ester in anaesthetized rats. Pharmacol Res, in press.
10. Malaisse WJ, Ladrière L, Laghmich A, Louchami K, Jijakli H, Viñambres C, Villanueva-Peñacarrillo ML, Valverde I and Björkling F, Insulinotropic action of 1,2,3-tri(methylsuccinyl)-glycerol ester. *Biochem Mol Med* **62:** 76–84, 1997.
11. Malaisse-Lagae F and Malaisse WJ, Insulin release by pancreatic islets. In: *Methods in Diabetes Research* (Eds. Larner J and Pohl SL), Vol. I, pp. 147–152. John Wiley and Sons, New York, 1984.
12. Levy J, Herchuelz A, Sener A, Malaisse-Lagae F and Malaisse WJ, Cytochalasin B-induced impairment of glucose metabolism in islets of Langerhans. *Endocrinology* **98:** 429–437, 1976.
13. Ladrière L, Laghmich A, Malaisse-Lagae F, Dannacher H, Björkling F and Malaisse WJ, Comparison between the insulinotropic potential of ten new esters of succinic acid. Eur J Pharmacol, in press.
14. Levy J and Malaisse WJ, The stimulus-secretion coupling of glucose-induced insulin release. XVII. Effects of sulfonylurea and diazoxide on insular biosynthetic activity. *Biochem Pharmacol* **24:** 235–239, 1975.
15. Viñambres C, Garcia-Martinez JA, Villanueva-Peñacarrillo ML, Valverde I and Malaisse WJ, Preservation of nutrient-

stimulated biosynthetic activity in pancreatic islets exposed to a meglitinide analogue. *Med Sci Res* **23:** 779–780, 1995.
16. Viñambres C, Villanueva-Peñacarrillo ML, Valverde I and Malaisse WJ, Repaglinide preserves nutrient-stimulated biosynthetic activity in rat pancreatic islets. *Pharmacol Res* **34:** 83–85, 1996.
17. Pipeleers DG, Marichal M and Malaisse WJ, The stimulus-secretion coupling of glucose-induced insulin release. XIV. Glucose regulation of insular biosynthetic activity. *Endocrinology* **93:** 1001–1011, 1973.
18. Hutton JC, Sener A, Herchuelz A, Atwater I, Kawazu S, Boschero AC, Somers G, Devis G and Malaisse WJ, Similarities in the stimulus-secretion coupling mechanisms of glucose- and 2-keto acid-induced insulin release. *Endocrinology* **106:** 203–219, 1980.
19. Malaisse WJ, Lebrun P, Yaylali B, Camara J, Valverde I and Sener A, Ketone bodies and islet function: ^{45}Ca handling, insulin synthesis and release. *Am J Physiol* **259:** E117–E122, 1990.
20. Sener A, Conget I, Rasschaert J, Leclercq-Meyer V, Villanueva-Peñacarrillo ML, Valverde I and Malaisse WJ, Insulinotropic action of glutamic acid dimethyl ester. *Am J Physiol* **267:** E573–E584, 1994.
21. MacDonald MJ and Fahien LA, Glyceraldehyde phosphate and methyl esters of succinic acid. Two "new" potent insulin secretagogues. *Diabetes* **37:** 997–999, 1988.
22. Fahien LA, MacDonald MJ, Kmiotek EH, Mertz RJ and Fahien CM, Regulation of insulin release by factors that also modify glutamate dehydrogenase. *J Biol Chem* **263:** 13610–13614, 1988.
23. Macdonald MJ and Fahien LA, Insulin release in pancreatic islets by a glycolytic and Krebs cycle intermediate: Contrasting patterns of glyceraldehyde phosphate and succinate. *Arch Biochem Biophys* **279:** 104–108, 1990.