BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **236,** 26–28 (1997)
ARTICLE NO. RC976901

# Metabolism of Glycerol-1,2,3-trimethylsuccinate in Rat Pancreatic Islets

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Received May 30, 1997

**The metabolism of $^{14}$C-labelled glycerol-1,2,3-trimethylsuccinate (2.0 mM) was examined in rat pancreatic islets. The oxidation of the glycerol moiety of the ester was negligible relative to that of its succinate residues. The oxidation of glycerol-1,2,3-trimethyl[1,4-$^{14}$C]succinate was two times higher than that of glycerol-1,2,3-trimethyl[2,3-$^{14}$C]succinate, this difference being matched by a higher generation of $^{14}$C-labelled acidic metabolites and amino acids from the latter than from the former tracer. The total generation of $^{14}CO_2$ from the ester, uniformly labelled except in its methyl groups, was close to that found for the oxidation of 1.0 mM D-[U-$^{14}$C]glucose. These findings thus reveal that glycerol-1,2,3-trimethylsuccinate is efficiently metabolized in islet cells and support the idea that this ester could be used as a nutrient to bypass defects of D-glucose transport and metabolism in the islet B-cell and, hence, improve proinsulin biosynthesis and insulin release in non-insulin-dependent diabetes mellitus.** © 1997 Academic Press

Selected esters of succinic acid are currently under investigation as possible insulinotropic tools in the treatment of non-insulin-dependent diabetes mellitus (1). One of the major potential objections to this novel therapeutic approach, namely the high concentrations or amounts of the esters required to stimulate insulin release *in vitro* or *in vivo*, was recently overcome by the design of new esters with high insulinotropic efficiency (2). The major aim of the present study is to investigate whether the insulinotropic potential of one of these new esters, *i.e.* glycerol-1,2,3-trimethylsuccinate is indeed commensurate with its capacity to act as a nutrient in rat pancreatic islets.

## MATERIALS AND METHODS

Unlabelled glycerol-1,2,3-trimethylsuccinate were prepared by direct esterification of glycerol by monomethyl succinic ester chloride, using a previously reported procedure (Patent application UK 9612331.0.1996). Radioactive glycerol-1,2,3-trimethylsuccinate was prepared by mixing glycerol (1 equiv., labelled or unlabelled), monomethyl succinate (4 or 9 equiv., labelled or unlabelled, respectively), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (6 or 10 equiv., respectively), 4-(dimethylamino)-pyridine (1 equiv.) in dichloromethane (dry, 0.2 ml/mg glycerol) at 0°C for 1 h and at 20°C for 1 h followed by mixing with 1,2-diaminoethane (13 equiv.) at 20°C for 1 h. Labelled glycerol-1,2,3-trimethylsuccinate was isolated by addition of ethyl acetate, washed with diluted hydrochloric acid, saturated aqueous sodium hydrogenocarbonate and brine, followed by chromatography on silica gel with ethyl acetate as eluant. Labelled monomethyl succinate was prepared by reacting labelled succinic anhydride in methanol (3 mg/ml) at 65°C for 150 min. [U-$^{14}$C]Glycerol (148 mCi/mmol; DuPont NEN, Boston, MASS), [1,4-$^{14}$C]succinic anhydride (1.7 mCi/mmol) and [2,3-$^{14}$C]succinic anhydride (2.1 mCi/mmol) both from Sigma (St. Louis, MO) were used in the synthesis of the radioactive ester. D-[U-$^{14}$C]Glucose (265 mCi/mmol) was obtained from DuPont NEN.

All experiments were conducted in groups of 20 islets each isolated by the collagenase procedure (3) from the pancreas of fed female Wistar rats (Proefdierencentrum, Heverlee, Belgium) and incubated for 120 min at 37°C in 40 $\mu$l of a bicarbonate-buffered medium (3) containing bovine serum albumin (5 mg/ml) and, as required, 2.0 mM glycerol-1,2,3-trimethylsuccinate, mixed with a tracer amount of either [U-$^{14}$C]glycerol-1,2,3-trimethylsuccinate (10 $\mu$Ci/ml), glycerol-1,2,3-trimethyl[1,4-$^{14}$C]succinate (10 $\mu$Ci/ml) or glycerol-1,2,3-trimethyl[2,3-$^{14}$C]succinate (7 $\mu$Ci/ml), or 1.0 mM D-glucose mixed with a tracer amount of D-[U-$^{14}$C]glucose (10 $\mu$Ci/ml). The methods used to measure the generation of $^{14}CO_2$ (4), $^{14}$C-labelled acidic metabolites (5) and amino acids (6) from the radioactive exogenous nutrients were identical to those described in the cited references. The blank values for the production of $^{14}CO_2$ from [U-$^{14}$C]glycerol-1,2,3-trimethylsuccinate, glycerol-1,2,3-trimethyl[1,4-$^{14}$C]succinate, glycerol-1,2,3-trimethyl[2,3-$^{14}$C]succinate and D-[U-$^{14}$C]glucose averaged, respectively, 0.02 ± 0.02, 0.12 ± 0.09, 0.12 ± 0.09 and 0.07 ± 0.01‰ of the total radioactive content of the incubation medium (n = 3 in all cases). The corresponding blank values for the generation of $^{14}$C-labelled acidic metabolites averaged 6.08 ± 1.17, 1.77 ± 0.87, 1.36 ± 0.58 and 0.32 ± 0.06%, and for the production of $^{14}$C-labelled amino acids 0.79 ± 0.09, 0.14 ± 0.06, 0.14 ± 0.05 and 0.02 ± 0.01%.

All results, including those already mentioned, are presented as mean values (±SEM), together with either the number of individual observations (n) or degree of freedom (d.f.). The statistical significance of differences between mean values was assessed by use of Student's t-test.

## RESULTS

The oxidation of [U-$^{14}$C] glycerol-1,2,3-trimethylsuccinate (2.0 mM) averaged 1.72 ± 0.07 pmol of ester

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0006-291X/97 $25.00

**TABLE 1**

Metabolism of Glycerol-1,2,3-Trimethylsuccinate and D-Glucose in Rat Pancreatic Islets

| Nutrient (mM) | $^{14}CO_2$[a] | $^{14}$C-Labeled acidic metabolites[a] | $^{14}$C-Labeled amino acids[a] |
|---|---|---|---|
| [U-$^{14}$C]glycerol-1,2,3-trimethylsuccinate (2.0) | 1.72 ± 0.07 (24) | N.D.[b] | N.D. |
| Glycerol-1,2,3-trimethyl[1,4-$^{14}$C]succinate (2.0) | 21.76 ± 2.13 (24) | 116.74 ± 15.76 (16) | N.D. |
| Glycerol-1,2,3-trimethyl[2,3-$^{14}$C]succinate (2.0) | 10.22 ± 0.80 (24) | 135.27 ± 15.78 (24) | N.D. |
| D-[U-$^{14}$C]glucose (1.0) | 9.45 ± 0.88 (24) | 25.70 ± 3.42 (24) | 5.13 ± 0.59 (24) |

[a] All results are expressed as pmol of nutrient equivalent/islet per 120 min incubation.
[b] N.D., not determined.

equivalent/islet per 120 min (n = 24). However, the readings for the net production of $^{14}$C-labelled acidic metabolites and amino acids were either not significantly different or, on occasion, somewhat lower than the blank value measured in media that had been incubated in the absence of pancreatic islets (Table 1).

The acetyl residues of glycerol-1,2,3-trimethylsuccinate were more efficiently oxidized than the glycerol part of the ester. This coincided with a large production of $^{14}$C-labelled acidic metabolites from either glycerol-1,2,3-trimethyl[1,4-$^{14}$C]succinate or glycerol-1,2,3-trimethyl[2,3-$^{14}$C]succinate. No sizeable generation of $^{14}$C-labelled amino acids from the latter two radioactive nutrients could be detected, when comparing the results obtained in the presence and absence of pancreatic islets.

The generation of $^{14}CO_2$ from glycerol-1,2,3-trimethyl[1,4-$^{14}$C]succinate was twice higher ($P < 0.001$) than that from glycerol-1,2,3-trimethyl[2,3-$^{14}$C]succinate. Inversely, the mean value for the net production of $^{14}$C-labelled acidic metabolites was slightly higher when the ester was labelled on the $C_2$ and $C_3$ of the succinate residues rather than on their $C_1$ and $C_4$ atoms. However, such a difference only achieved statistical significance ($P < 0.025$) by comparison of the results recorded within each individual experiment. The mean value for the $^{14}$C-labelled amino acid content of media obtained after incubation of islets in the presence of glycerol-1,2,3-trimethyl[2,3-$^{14}$C]succinate also exceeded ($P < 0.001$) by 5.17 ± 1.23 pmol of ester equivalent/islet per 120 min (d.f. = 42) the corresponding value found, within the same experiments, after exposure of the islets to glycerol-1,2,3-trimethyl[1,4-$^{14}$C]succinate.

For purpose of comparison, the metabolism of D-[U-$^{14}$C]glucose (1.0 mM) was also examined in these experiments. The concentration of the hexose was selected to match, in terms of triose residues and on a molar basis, that of the glycerol moiety of the ester. Expressed as $^{14}CO_2$, the oxidation of D-[U-$^{14}$C]glucose (56.7 ± 5.3 pmol/islet per 120 min) was about ten times higher, however, than that of [U-$^{14}$C] glycerol-1,2,3-trimethylsuccinate (5.2 ± 0.2 pmol/islet per 120 min). As expected, at the low concentration of D-glucose used in the present experiments, the production of $^{14}$C-labelled acidic metabolites from the uniformly labelled hexose exceeded ($P < 0.005$) its oxidation and conversion to radioactive amino acids.

## DISCUSSION

The present work documents the hydrolysis of glycerol-1,2,3-trimethylsuccinate and the further catabolism of its glycerol and succinate parts (Fig. 1).

The conversion of [U-$^{14}$C]glycerol-1,2,3-trimethylsuccinate to $^{14}CO_2$ occurred at a rate not exceeding that found with exogenous [U-$^{14}$C]glycerol tested at the same extracellular concentration (2.0 mM) as the ester (7). No sizeable generation of $^{14}$C-labelled acidic metabolites and amino acids from [U-$^{14}$C]glycerol-1,2,3-trimethylsuccinate could be detected by the ion exchange chromatography procedure here used for their measurement.

From the quantitative standpoint, the major product of glycerol-1,2,3-trimethylsuccinate metabolism consisted in radioactive acidic metabolites formed from the ester when labelled in its succinyl residues. Such metabolites are likely to correspond, for their major part, to succinic acid itself and its mono-esters generated by hydrolysis of glycerol-1,2,3-trimethylsuccinate.

The oxidation of the succinate residues of glycerol-1,2,3-trimethylsuccinate was about one order of magni-

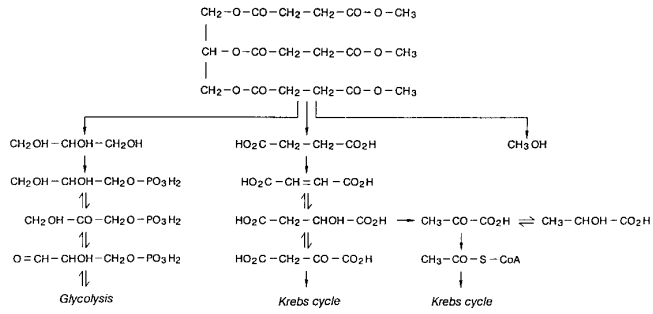


**FIG. 1.** Schematic view of the metabolism of glycerol-1,2,3-trimethylsuccinate.

tude lower than their net generation from the ester. As expected from a prior study dealing with the metabolic fate of the dimethyl ester of succinic acid in rat pancreatic islets (8), the production of $^{14}CO_2$ from the $C_1$ and $C_4$ of the succinate residues in glycerol-1,2,3-trimethylsuccinate largely exceeded that from the corresponding $C_2$ and $C_3$ atoms. This difference in $^{14}CO_2$ output was matched by complementary changes in the generation of $^{14}$C-labelled amino acids and acidic metabolites. The higher yield of $^{14}CO_2$ from glycerol-1,2,3-trimethylsuccinate than from glycerol-1,2,3-trimethyl[2,3-$^{14}$C]-succinate may be attributed to two mechanisms. First, when deesterified and metabolized through the sequence of reactions catalyzed by succinate dehydrogenase, fumarase, the malic enzyme and pyruvate dehydrogenase, each molecule of [1,4-$^{14}$C]succinate generates two molecules of $^{14}CO_2$, whereas [2,3-$^{14}$C]succinate is converted to [*acetyl*-1,2-$^{14}$C]CoA without any production of $^{14}CO_2$. Likewise for each molecule of $^{14}$C-labelled pyruvate generated from radioactive succinate and then converted to either L-lactate or L-alanine, one molecule of $^{14}CO_2$ is produced from [1,4-$^{14}$C]succinate but none from [2,3-$^{14}$C]succinate. Second, when $^{14}$C-labelled malate generated from [1,4-$^{14}$C]succinate circulates in the Krebs cycle, all $^{14}$C atoms are converted to $^{14}CO_2$ during the first turn of the cycle. However, when $^{14}$C-labelled malate is derived from [2,3-$^{14}$C]-succinate and circulates in the Krebs cycle, no $^{14}CO_2$ is formed during the first turn of the cycle and, at the occasion of each further turn, only 50% of the residual $^{14}$C atoms are converted to $^{14}CO_2$.

The total generation of $^{14}CO_2$ from 2.0 mM glycerol-1,2,3-trimethylsuccinate, uniformly labelled with $^{14}$C except in the methyl groups presumably converted to methanol, amounted to 69.1 ± 4.5 pmol/islet per 120 min, a value close to that found for the generation of $^{14}CO_2$ from 1.0 mM D-[U-$^{14}$C]glucose (56.7 ± 5.3 pmol/islet per 120 min). Yet, the insulinotropic action of 2.0 mM glycerol-1,2,3-trimethylsuccinate largely exceeds that of 1.0 mM D-glucose (2). Since the respiratory quotients for the full oxidation of either the hexose or the ester, after its hydrolysis to liberate methanol, are both close to unity (1.00 for D-glucose and 1.07 for glycerol-1,2,3-trimethylsuccinate), the comparison between the oxidative and secretory data could suggest that, relative to ATP generation rate, glycerol-1,2,3-trimethylsuccinate is a more potent insulin secretagogue than D-glucose. It should be realized, however, that our metabolic data do not inform on the possible interferences of the two secretagogues with the catabolism of endogenous nutrients in the islet cells. In a prior study, both the monomethyl and dimethyl ester of succinic acid were found to decrease $^{14}CO_2$ output from islets prelabelled with [U-$^{14}$C]palmitate (8). Such a sparing action may well account for a lesser increase in total $O_2$ uptake than suggested by the data here collected in islets exposed to $^{14}$C-labelled glycerol-1,2,3-trimethylsuccinate. Indeed, since the oxidation of endogenous fatty acids accounts for a major fraction of basal respiration in the islets (9), it remains quite possible that the insulinotropic action of glycerol-1,2,3-trimethylsuccinate and that of D-glucose, which fails to affect $^{14}CO_2$ output from islets prelabelled with [U-$^{14}$C]palmitate when tested at the low concentration used in the present experiments (10), are both commensurate with their capacity to act as fuels in the islet cells. Moreover, in islets exposed to glycerol-1,2,3-trimethylsuccinate, the coupling of metabolic to functional events might be affected by factors such as a lowering of intracellular pH due to the hydrolysis of the ester (unpublished observation).

In conclusion, therefore, this study reveals that glycerol-1,2,3-trimethylsuccinate is efficiently metabolized in islet cells and, hence, reinforces the view that this ester could be used as an alternative nutrient to bypass site-specific defects of D-glucose transport and metabolism in the B-cell and, by doing so, improve ATP generation, proinsulin biosynthesis and insulin release in non-insulin-dependent diabetes mellitus.

## ACKNOWLEDGMENTS

This study was supported by a grant (3.4578.92) from the Belgian Foundation for Scientific Medical Research and a Concerted Research Action (94/99-183) of the French Community of Belgium. We are grateful to M. Mahy for technical assistance and C. Demesmaeker for secretarial help.

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