Effects of aliphatic dioic acids and glycerol-1,2,3-tris(dodecanedioate) on D-glucose-stimulated insulin release in rat pancreatic islets

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Aliphatic dioic acids have been proposed as alternative nutrients in selected clinical situations. In this study, their possible insulinotropic action was investigated in isolated rat pancreatic islets prepared from fed rats. Azelaic acid, sebacic acid and tridecanedioic acids, when tested at a 10·0 mM concentration, were found to augment insulin release evoked by D-glucose (7·0 mM) in the pancreatic islets. Likewise, glycerol-1,2,3-tris(dodecanoedioate), when used at concentrations close to 1·0 mM, increased the secretory response to the hexose. It is speculated that these findings may extend to insulin-producing cells, the knowledge that aliphatic dioic acids or their esters may act as energy substrates, e.g. in parenteral nutrition.

Medium-chain dicarboxylic acids have been recently regarded as possible alternate fuel substrates both in normal and pathological conditions in man (Mingrone *et al.* 1989, 1992, 1996; Tacchino *et al.* 1990; Bertuzzi *et al.* 1991). The energy densities of these compounds (20·79 kJ/g (4·97 kcal/ g) for azelaic acid, C9, 27·78 kJ/g (6·64 kcal/g) for sebacic acid, C10, and 30·12 kJ/g (7·2 kcal/g) for dodecanedioic acid, C12) are approximately in-between the values known for the conventional energy substrates, such as glucose (15·69 kJ/g (3·75 kcal/g)) and fatty acids (37·67 kJ/g (9 kcal/g)). A major drawback of these dioic acids is the relatively high degree of urinary loss which is proportionally lower as their chain length increases (Bertuzzi *et al.* 1991; Mingrone *et al.* 1996). The triacylglycerol of dodecanedioic acid, however, while preserving its water solubility appears to have the lowest urinary excretion in experimental animals, corresponding to approximately 0·67 % of the administered amount. Kinetics studies in rats (De Gaetano *et al.* 1999) have shown that this triacylglycerol has a large volume of distribution (approximately 0·5 l/kg body weight) with a fast disappearance rate from plasma (0·42 min^{-1}), whereas one of its product of hydrolysis, dodecanedioic acid, has a very small volume of distribution (approximately 0·04 l/kg body weight) and a high tissue uptake with maximal transport rate of 0·636 mmol/min. The measured dodecanedioic acid tissue uptake rate is consistent with the possibility of achieving substantial energy delivery, should it be added to parenteral nutrition formulations. Finally, the amount of Na administered with the triacylglycerol form is one-half of that necessary for water solubility with the free diacid.

It has been demonstrated, by using euglycaemic hyperinsulinaemic clamp and indirect calorimetry, that medium-chain dicarboxylic acids can compete with glucose for cellular utilization in human subjects (Raguso *et al.* 1994; Mingrone *et al.* 1997). A direct effect of these acids on pancreatic insulin delivery has not been investigated up to now. The purpose of the present investigation is to study the effects of these water-soluble nutrients and one of their esters (C12 triacylglycerol) on glucose-stimulated insulin release in isolated rat pancreatic islets.
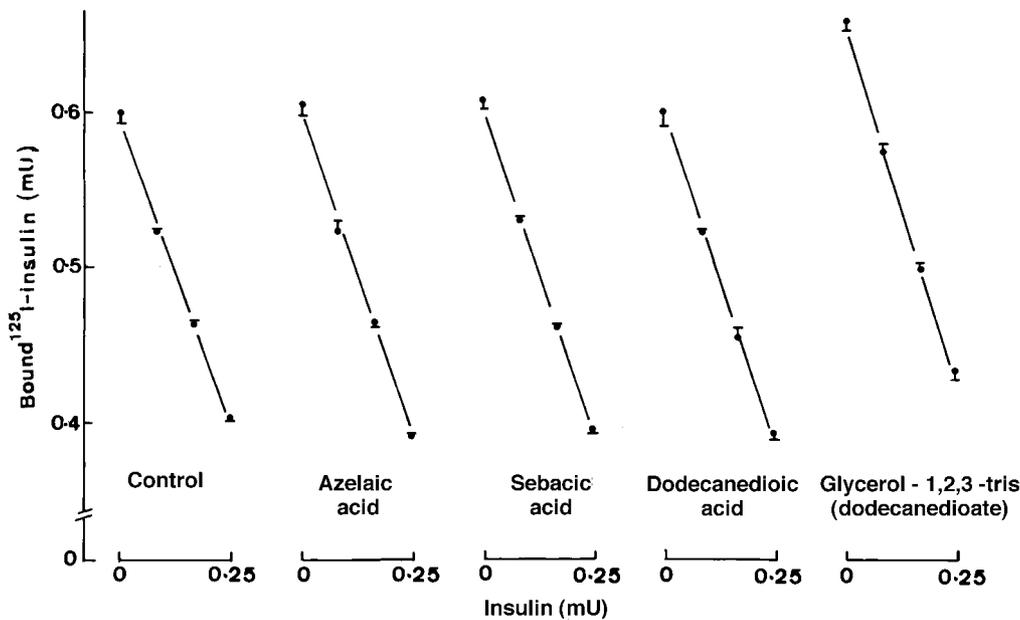
Materials and methods

All tested medium-chain fatty acids were obtained from Sigma-Tau (Pomezia, Italy).

Pancreatic islets were isolated by the collagenase procedure (Malaisse-Lagae & Malaisse, 1984) from female Wistar rats (B & K Limited, Hull, E. Yorks., UK) given free access to food (KM-04-k12; Pavan Service, Oud Turnhout, Belgium) and tap water up to the time of killing by decapitation.

Insulin secretion was measured over a 90 min incubation at 37°C in groups of eight freshly isolated islets each placed in 1·0 ml bicarbonate-buffered medium (Malaisse-Lagae & Malaisse, 1984) containing bovine serum albumin (5 mg/ ml), D-glucose (7·0 or 11·1 mM) and, as required, an aliphatic dioic acid or glycerol-1,2,3-tris(dodecanedioate) (1·0–10·0 mM).

Since medium-chain fatty acids may interfere with the radioimmunoassay of insulin (Malaisse & Malaisse-Lagae,

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Fig. 1. Binding of ^{125}I-insulin by guinea-pig anti-insulin serum, which was preincubated for 30 min at 37°C in the presence of increasing amounts of unlabelled insulin (abscissae), the assay medium containing, as required, azelaic acid, sebacic acid, dodecanedioic acid or glycerol-1,2,3-tris(dodecanedioate) at the same final concentration as that used for measuring insulin release from islets incubated in the presence of $10 \cdot 0$ mM aliphatic dioic acids and $4 \cdot 0$ mM glycerol ester. Values are means with standard errors of the means for four estimations. For such a measurement, aliquots ($0 \cdot 2$ ml) of the incubation medium are mixed with $0 \cdot 8$ ml phosphate buffer ($0 \cdot 1$ M, pH $7 \cdot 0$) containing diluted guinea-pig anti-insulin serum and, after 30 min incubation at 37°C, exposed for another period of 30 min at 20°C to a mixture of unlabelled bovine and ^{125}I-labelled porcine insulin. Excess unbound insulin is then removed by adsorption to cellulose.

1968), control experiments were performed to assess the possible effect of four of the tested fatty acids on the back-titration procedure used for the measurement of insulin released by the islets (Wright *et al.* 1967). As shown in Fig. 1, the results obtained in the presence of azelaic, sebacic and dodecanoic acids were undistinguishable from the control data. Glycerol-1,2,3-tris(dodecanedioate), however, increased the amount of bound ^{125}I-insulin, without affecting either the blank value measured in the absence of guinea-pig anti-insulin serum (data not shown) or the decrease in ^{125}I-insulin binding attributable to preincubation of the guinea-pig antibodies with unlabelled insulin (Fig. 1). In these control experiments, the concentration of the tested agents was identical to that used in the measurement of insulin release. Pooling all available data, the paired ratio of ^{125}I-insulin binding in the presence or absence of glycerol-1,2,3-tris(dodecanedioate):guinea-pig antibodies preincubated in the absence of exogenous insulin averaged $108 \cdot 8$ (SEM $0 \cdot 6$) % (n 5, $P < 0 \cdot 001$ as compared to unity).

All results, including those mentioned earlier, are expressed as means with standard errors of the means together with the number of individual observations (n). The statistical significance of differences between mean values was assessed by use of Student's t test.

Results

In the presence of $7 \cdot 0$ mM D-glucose, both azelaic and sebacic acids ($10 \cdot 0$ mM each) augmented insulin output ($P < 0 \cdot 001$), whereas dodecanedioic acid ($10 \cdot 0$ mM) failed to do so (Table 1). Comparable results were obtained at a higher concentration of D-glucose ($11 \cdot 1$ mM). Thus, azelaic acid ($10 \cdot 0$ mM) again augmented insulin secretion ($P < 0 \cdot 03$), whilst the increment in insulin output attributable to sebacic acid and dodecanedioic acid failed to achieve statistical significance.

When tested at lower concentrations ($1 \cdot 0$ to $4 \cdot 0$ mM), azelaic acid, sebacic acid and dodecanedioic acid all failed to augment significantly insulin output evoked by $7 \cdot 0$ mM-D-glucose (Table 2). However, in this low range of concentrations, glycerol-1,2,3-tris(dodecanedioate) augmented glucose-stimulated insulin release. The most pronounced effect of the triacylglycerol was recorded at a concentration of $1 \cdot 0$ mM, when the output of insulin averaged $158 \cdot 1$ (SEM $14 \cdot 3$) % (n 19, $P < 0 \cdot 005$) of the mean corresponding control value ($100 \cdot 0$ (SEM $11 \cdot 1$) %; n 20). In this second series of experiments, the release of insulin measured in the sole presence of $7 \cdot 0$ mM D-glucose averaged $51 \cdot 8$ (SEM $7 \cdot 1$) μU/islet per 90 min (n 75).

In the last series of experiments, which were again conducted in the presence of $7 \cdot 0$ mM-D-glucose, further aliphatic dioic acids with 3–14 C atoms were tested in the range of $1 \cdot 0$–$10 \cdot 0$ mM (Table 3). In some cases, the highest of these concentrations ($10 \cdot 0$ mM) was not tested, because it had already been examined in prior publications (succinic acid; see Malaisse *et al.* 1993) or failed to yield a clear solution in the bicarbonate-buffered medium (tetradecanedioic acid). In this last set of experiments, the secretion of insulin recorded in the sole presence of D-glucose averaged $48 \cdot 8$ (SEM $3 \cdot 5$) μU/islet per 90 min (n 123). Malonic acid,

Table 1. Effects of azelaic acid, sebacic acid and dodecanedioic acid on insulin release evoked by 7·0 and 11·1 mM-D-glucose*

(Mean values with standard errors of the means)

| | Insulin output (μU/islet per 90 min) | | | | | |
| | D-glucose (7·0 mM) | | | D-glucose (11·1 mM) | | |
Tested agent	Mean	SEM	n	Mean	SEM	n
Nil	41·1	3·8	24	247·9	17·6	24
Azelaic acid (10·0 mM)	66·8	5·9	24	301·5	15·2	24
Sebacic acid (10·0 mM)	67·9	4·5	23	271·3	11·6	24
Dodecanedioic acid (10·0 mM)	46·3	3·4	24	271·6	16·9	24

* For details of procedures see p. 733.

succinic acid, pimelic acid and undecanedioic acid all failed to affect insulin release significantly. Tridecanedioic acid augmented glucose-stimulated insulin release ($P < 0.05$) when tested at a 10·0 mM concentration, but not at lower concentrations. Only tetradecanedioic acid inhibited ($P < 0.005$ or less) the secretory response to D-glucose.

Discussion

The role of the interaction between glucose metabolism and intracellular fatty acids disposition in coupling stimulation and secretion of insulin from β-cells has been largely recognized (Greenough *et al.* 1967; Malaisse & Malaisse-Lagae, 1968; Campillo *et al.* 1979; McGarry & Dobbins, 1999). A common element in the glucose–fatty acid metabolism pathways is the malonyl-CoA molecule (Ruderman *et al.* 1999), whose concentration is thought to modulate the access of these energy substrates to the Krebs cycle.

Glycolysis allows the formation of pyruvate, which can be converted to citrate in mitochondria. While a fraction of this citrate is oxidized in the tricarboxylic-acid cycle to CO_2 and H_2O generating ATP, the remainder can leave the mitochondria to be converted into malonyl-CoA.

The increase in malonyl-CoA cytosolic level, by suppressing the carnitine palmitoyltransferase I activity, determines an increase in the cytosolic concentration of long-chain acyl-CoA. This last event may act as a signal for insulin secretion.

The β-oxidation of one mol odd-numbered C atom dicarboxylic acid produces, together with a certain number of acetyl-CoA mol proportional to the dicarboxylic acid chain length, one mol malonyl-CoA, while even-numbered dicarboxylic acids allow formation of succinyl-CoA in

addition to acetyl-CoA. It is likely, therefore, that odd-numbered dicarboxylic acids can stimulate insulin release from pancreatic β-cells in the presence of glucose. We indeed observed that, in presence of 7·0 mM-D-glucose, azelaic acid (C9) causes a 62 % increase in insulin output at a concentration of 10 mM, while this effect is diminished to about half (35 %) as its concentration is reduced ten-fold (1 mM). The effect of sebacic acid (C10) was similar to that of C9 at concentrations of 10 mM, while at 1 mM C10 increased insulin output by only 17 %; such an increase failed to achieve statistical significance. Among the other nutrients tested, 1 mM glycerol triester of dodecanedioic acid produced the largest stimulation of β-cell insulin release (58 % greater than control).

The similar effect of C9 and C10 suggests that both malonyl-CoA and succinyl-CoA, which are the end-products of the β-oxidation of these two dicarboxylic acids respectively, produce a quantitatively similar stimulatory effect on insulin release. These results are also in accordance with previous observations (Malaisse *et al.* 1993; Ladrière *et al.* 1999) that the esters of succinic acid show a high insulinotropic efficiency both *in vitro* and *in vivo*.

It may appear surprising that dodecanedioic acid (C12), which has the same metabolic pathway as C10, did not show the same degree of effect when tested at a 10 mM concentration. This might be explained by the different water solubility of the two diacids, which implies that at this concentration a lower amount of C12 can enter the β-cells to be β-oxidized. In fact, at a 1·0 mM concentration the effects of C10 and C12 were comparable. Tetradecanediodic acid inhibited glucose-stimulated insulin release. The determinants of this unexpected situation remain to be elucidated.

As far as the triacylglycerol of C12 is concerned, the higher stimulatory effect on insulin release at 1 mM

Table 2. Effects of low concentrations of azelaic acid, sebacic acid, dodecanedioic acid and its ester on insulin release evoked by 7·0 mM-D-glucose*

(Mean values with standard errors of the means)

| | 0·0 mM | | | 1·0 mM | | | 4·0 mM | | |
Diacid (ester)	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n
Azelaic acid	100·0	20·7	15	135·0	26·3	18	134·0	17·4	19
Sebacic acid	100·0	14·6	16	117·5	15·3	18	111·0	15·0	18
Dodecanedioic acid	100·0	8·0	24	113·5	11·1	24	114·3	9·0	24
Glycerol-1,2,3-tris(dodecanedioate)	100·0	11·1	20	158·1	14·3	19	132·6	9·9	19

* All results are expressed as a percentage of the mean value recorded within the same experiment(s) in the sole presence of D-glucose. For details of procedures see p. 733.

Table 3. Effects of aliphatic dioic acids on insulin release evoked by 7·0 mM-D-glucose*

(Mean values and standard errors of the means)

	0·0 mM			1·0 mM			2·0 mM			4·0 mM			10·0 mM		
	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n	Mean	SEM	n
Malonic acid	100·0	28·4	17	94·1	14·8	19	72·1	10·2	19	91·8	17·9	14	111·5	15·9	18
Succinic acid	100·0	25·6	15	114·2	19·3	17	84·5	25·9	18	76·1	20·0	18	ND		
Pimelic acid	100·0	8·7	19	113·6	10·1	18	105·8	9·0	20	95·5	9·0	20	ND		
Undecanedioic acid	100·0	12·5	24	93·2	10·9	24	99·5	7·9	24	115·8	9·2	24	100·5	11·4	24
Tridecanedioic acid	100·0	12·1	24	86·6	9·9	24	105·8	20·6	24	95·9	9·6	24	141·4	15·3	24
Tetradecanedioic acid	100·0	10·0	24	52·2	6·3	24	48·8	12·1	24	28·1	3·5	24	ND		

ND, not determined.

* All results are expressed as a percentage of the mean value recorded within the same experiment(s) in the sole presence of D-glucose. For details of procedures see p. 733.

concentration might be ascribed to a bypass phenomenon of dicarboxylic acid-specific receptors with a passive transport by diffusion across the cellular membrane, like that hypothesized for glucose esters (Malaisse, 1999). It is also likely that, once one molecule C12 triacylglycerol enters the β-cells, it must be hydrolysed to one molecule glycerol and three molecules dodecanedioic acid. The higher amount of C12 supplied to the cells could thus explain the higher stimulatory effect on insulin release.

In conclusion, the present study showing a direct effect of dicarboxylic acids on insulin-producing cells further supports the contention that medium-chain dicarboxylic acids can be regarded as useful energy substrates. This holds true also for glycerol-1,2,3-tris(dodecanedioate), at least when this triacylglycerol is used at a low concentration. These findings are obviously relevant to the possible use of these alternative nutrients in clinical situations, such as decompensated diabetes mellitus (Greco & Mingrone, 1995; Mingrone *et al.* 1997).

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