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Review Article

Insulinotropic nutrient esters

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Introduction

The design of new insulinotropic agents for the treatment of noninsulin-dependent diabetes mellitus is a very active field of drug research. It is currently believed that in type-2 diabetes impaired insulin secretion contributes to the perturbation of glucose homeostasis. In this disease, the altered secretory behavior of the endocrine pancreas may result from either inherited or acquired primary defects. It may also involve a secondary phenomenon of so-called B cell incompetence that is the consequence of sustained hyperglycemia or other metabolic anomalies (*e.g.*, hyperlipacidemia). In many, but not all, type-2 diabetic patients, the pancreatic islet B cells display a preferential perturbation of their secretory response to D-glucose, as compared to other nutrient secretagogues or nonnutrient insulinotropic agents. This type of "blindness" to D-glucose may affect the insulinotropic action of antidiabetic drugs. In normal B cells, the magnitude of the B cell functional response to such compounds as hypoglycemic sulfonylureas, meglitinide analogs or GLP-1 is tightly modulated by the ambient concentration of D-glucose or other nutrients. For instance, generally it is negligible in islets incubated in the absence of any exogenous nutrient and much more pronounced in islets exposed to insulinotropic concentrations of D-glucose. It is conceivable, therefore, that the impaired metabolism of D-glucose and, in some cases, of other circulating nutrients in the B cells of type-2 diabetic patients prevents the insulin-releasing drugs used in treating these patients to fully exhibit their insulinotropic potential.

It was speculated, therefore, that nutrients which could avoid the site-specific metabolic defects responsible for the impaired B cell response to D-glucose in non-insulin-dependent diabetes would represent useful tools both to stimulate insulin release *per se* in the diseased endocrine pancreas and to enhance the secretory response to antidiabetic drugs (1).

This novel therapeutic approach also offers a theoretical advantage over all other insulinotropic agents currently used in the treatment of type-2 diabetes, that is, stimulation of both proinsulin biosynthesis and insulin release. As distinct from nonnutrient secretagogues, virtually all nutrients that stimulate insulin release also increase the rate of proinsulin biosynthesis in isolated pancreatic islets.

The selection of a suitable nonglucidic nutrient secretagogue obviously is the key issue of this therapeutic approach. Inspired by the knowledge that the insulinotropic efficiency of nutrients appears to be closely related to their capacity to increase ATP generation in B cells, and considering the quantitatively key contribution of the mitochondrial Krebs cycle in ATP production, attention was first focused on dicarboxylic metabolites of the Krebs cycle or their immediate precursors as potential new nutrients. However, several of these metabolites, *e.g.*, succinic acid, do not penetrate easily into islet cells. In contrast, the esters of carboxylic metabolites were found to efficiently cross the B cell plasma membrane, where they then undergo intracellular enzymatic hydrolysis, so that the resulting acidic metabolite then becomes readily available for further catabolism.

The major aim of this article is to review the experimental information presently available on the use of such esters as possible insulinotropic tools for the treatment of type-2 diabetes. The recent extension of this approach to the esters of selected monosaccharides will also be briefly discussed.

Succinic acid methyl esters

The monomethyl ester of succinic acid (SAM) and its dimethyl ester (SAD) were the first agents evaluated for their insulinotropic potential in the design of new insulin-releasing nutrients (2, 3). Shortly after their introduction as insulin secretagogues (4, 5), they were found to increase O_2 uptake by rat islets incubated in the absence or presence of D-glucose; to decrease ^{86}Rb out-

flow from prelabeled islets; to stimulate biosynthetic activity in the islets, with a preferential effect on the synthesis of proinsulin; to inhibit ^{45}Ca efflux from prelabeled islets perifused in the absence of extracellular Ca^{2+} but to increase ^{45}Ca net uptake and to cause a biphasic stimulation of ^{45}Ca outflow in islets incubated or perifused in the presence of extracellular Ca^{2+}; and to evoke a biphasic stimulation of insulin release. The insulinotropic action of these methyl esters coincided with a shift to the left of the sigmoidal relationship between insulin output and D-glucose concentration; was concentration-related in the 2-10 mM range; failed to be duplicated by succinic acid; displayed both Ca^{2+} dependency and resistance to a lowering of extracellular pH; and was operative in the absence of D-glucose whether or not the islets were stimulated by nonnutrient secretagogues. It was concluded that the respiratory, cationic, biosynthetic and secretory responses of the islets to succinate methyl esters display the characteristic features usually found in the process of nutrient-stimulated insulin release (2).

The metabolic effects and the catabolism of succinate methyl esters were then examined in rat pancreatic islets. The esters increased $^{14}CO_2$ production from islets prelabeled with L-[U-^{14}C]glutamine but inhibited NH_4^+ output, suggesting that they do not activate glutamate dehydrogenase. They decreased $^{14}CO_2$ output from islets prelabeled with [U-^{14}C]palmitate. They had little effect on the oxidation of exogenous D-[3,4-^{14}C]glucose, D-[2-^{14}C]glucose, D-[6-^{14}C]glucose or D-[1-^{14}C]glucose, suggesting unaltered ratio between the input of acetyl residues and 4- or 5-carbon metabolites, such as succinate, into the Krebs cycle. By following the fate of both [1,4-^{14}C]succinate dimethyl ester and [2,3-^{14}C]succinate dimethyl ester, data were obtained indicating that succinate is efficiently formed from the ester and further metabolized, leading to the generation of ^{14}C-labeled acidic metabolites, including pyruvate and L-lactic acid, CO_2 and amino acids. It was proposed that an increase in both succinate and acetyl residue influx into the Krebs cycle accounts for the increase in O_2 uptake caused by the succinate methyl esters and, hence, the stimulation of proinsulin biosynthesis and insulin release (3).

In a further study, the hydrolysis of the dimethyl ester of [1,4-^{14}C]succinic acid and/or [2,3-^{14}C]succinic acid was measured in homogenates of rat pancreatic islets (6). Both particulate and cytosolic enzymatic activity were observed upon subcellular fractionation. The activity found in islet homogenates corresponded to the rate of SAD hydrolysis in intact cells. While the intracellular pool of acidic metabolites generated from SAD remained fairly stable over 15-120 min of incubation and was mainly located in the cytosolic compartment, the amount of acidic metabolites released in the extracellular milieu progressively increased with the duration of incubation. Such metabolites included monocarboxylic and dicarboxylic acids, the latter consisting mainly of succinic acid and, to a lesser extent, fumaric acid and malic acid. In contrast to SAD, succinic acid was not taken up by intact islets.

In a reverse hemolytic plaque assay for insulin secretion from single B cells, SAM (10.0 mM) increased the percentage of plaque-forming cells and mean value for plaque area in cells exposed to 5.0 mM L-leucine (7). Likewise, SAM (2-10 mM) increased the percent of plaque-forming cells and total plaque development in cells exposed to 5 mM 2-amino-bicyclo[2,2,1]heptane-2-carboxylic acid (BCH) in a concentration-dependent manner. In experiments including two successive incubations of 45 min each, the same B cells responded to either D-glucose or the combination of SAM (10 mM) and BCH (5 mM), with a few cells responding to only one of the two stimulations (8).

SAM and SAD also enhanced the B cell secretory response to various antidiabetic agents. In rat pancreatic islets, glibenclamide- or glimepiride-induced increases in insulin output were much higher in the presence of SAD than in its absence (9). Moreover, insulin secretion was higher in the presence of SAD (10.0 mM) and a hypoglycemic sulfonylurea (1.0 μM) than in the presence of D-glucose (6.0 mM) and the same sulfonylurea, despite the fact that in the absence of the hypoglycemic agent, the release of insulin caused by SAD was somewhat lower, albeit not significantly so, than that provoked by the hexose. Similarly, SAM (10.0 mM), like D-glucose (7.0 mM), allowed meglitinide and several of its analogs (S3075, A-4166, KAD-1229, repaglinide) to cause a pronounced increase in insulin output, while all these agents (10 μM) failed to significantly affect basal insulin release in rat pancreatic islets (10). A-4166 (10 μM) did not significantly affect the stimulant action of SAM (10 mM) on biosynthetic activity in rat pancreatic islets (11).

A-4166 (20 μM) increased insulin release from islets incubated in the presence of 10 mM SAM in rats injected with streptozotocin during the neonatal period (STZ rats) and in hereditarily diabetic Goto-Kakizaki rats (GK rats) (12). In these animal models of type-2 diabetes, the insulinotropic action of SAM and SAD was often impaired, especially in GK rats (12-14).

In another experimental model of B cell dysfunction in hyperglycemic rats infused for 48 h with a hypertonic solution of D-glucose (1.67 M), SAD (10 mM) markedly enhanced insulin release evoked by 16.7 mM D-glucose, alone or in combination with 0.5 μM glimepiride, in the isolated perfused pancreas preparation (15). In these experiments, similar results were found in pancreases prepared from control euglycemic rats.

SAD also potentiated the B cell secretory response to glucagon-like peptide-1 (GLP-1). While GLP-1 (1.0 nM) had no effect on insulin release from isolated rat pancreases perfused in the absence of an exogenous nutrient, it caused a biphasic enhancement of insulin release evoked by 10 mM SAD (16). This was documented in both normal and GK rats (16, 17). Thus, nonglucidic nutrients such as SAD may optimize the B cell secretory response to GLP-1 in noninsulin-dependent diabetes. Incidentally, when administered enterally to overnight fasted rats, SAD provoked a rapid and sustained increase in plasma GLP-1 concentration (18).

SAM and SAD were also found to stimulate insulin release *in vivo*. The intravenous administration of SAM and SAD (1.0 μmol/g) to fasted and anesthetized rats resulted in a greater increase in plasma insulin concentration than that evoked by D-glucose (1.1 μmol/g i.v.) 2 min after administration. The increase in plasma insulin concentration, relative to the molar amount of nutrient injected in the blood stream, averaged 12.8 ±. 1.5 ng/ml/μmol with succinic acid esters as compared to only 6.1 ± 1.0 ng/ml/μmol with D-glucose (19). Similarly, the intraperitoneal administration of SAM (2.2 μmol/g) increased the plasma insulin concentration, reaching a peak value about 30 min after administration (19).

Unexpectedly, even the intragastric administration of SAD (10 μmol/g) to conscious overnight fasted rats caused a rapid and marked increase in plasma insulin concentration; the effect was less pronounced with SAM and was absent with unesterified succinic acid (20, 21). Comparable results were obtained after intraduodenal administration of SAM or SAD (22).

As *in vitro*, SAM and SAD also enhanced the secretory response of insulin-producing cells to glibenclamide (23) and A-4166 (24) *in vivo*. The insulinotropic action of SAM (1.0 μmol/g), as well as its enhancing effect on the secretory response to glibenclamide (0.1 nmol/g), were severely impaired in adult STZ rats (25). Repeated intraperitoneal administration of SAM (1.0 μmol/g t.i.d.) for 7-10 days failed to significantly improve the insulin secretory response to D-glucose or glibenclamide in both control and STZ rats (25).

SAM (2.0-4.0 μmol/g i.p.), when injected to fed rats 15 min before the administration of streptozotocin (0.15 μmol/g i.v.), lowered (p <0.001) glycemia, measured 5 days later, from 28.1 ± 1.1 mM (rats injected with STZ alone) to 16.1 ± 1.9 mM, compared to 8.7 ± 0.2 mM in control (uninjected) rats (26). Thus, SAM and SAD may be useful in the treatment of noninsulin-dependent diabetes not only because of their insulinotropic effects but also because of their protective effects against cytotoxic processes such as those possibly involved in the secondary phenomenon of B cell glucotoxicity.

Further studies showed that higher insulin release in response to glucose and higher glucose oxidation rates were observed in rat pancreatic islets exposed to interleukin-1β (IL-1β) in the presence of SAM (10 mM), as compared to islets exposed to IL-1β alone. These beneficial effects of SAM were not accompanied by any decrease in IL-1β-induced nitric oxide (NO) production nor in the inhibition of aconitase activity. Moreover, SAM did not increase the biosynthesis of glutamate decarboxylase. Thus, SAM appeared to improve B cell function primarily by increasing the capacity of these cells to endure NO exposure and partial blockade of the Krebs cycle (27). This is in agreement with the fact that SAM (10 mM) failed to protect tumoral islet cells (RINm5F) against the effects of IL-1β on NO production, cell viability, [^{3}H]thymidine incorporation, D-[5-^{3}H]glucose utilization and D-[6-^{14}C]glucose oxidation (28). The discrepancy observed between normal and tumoral islet cells may be attributed in part to the vastly different contribution of mitochondrial oxidative events to overall ATP generation in these two cell types.

The capacity of SAM and SAD to act as nutrients is not restricted to islet cells. These esters were found to be efficiently metabolized and to exert a sparing action on the catabolism of D-glucose in hepatocytes (29), myocytes (30), neural cells (31), adipocytes (32), colon carcinoma cells and normal intestinal cells (33, 34). When the nutritional value of SAD in the whole organism was assessed by infusing the ester (80 μmol/g/day) for 2 or 4 days to starved rats, the decrease in plasma D-glucose concentration, liver glycogen content and hepatic glucokinase activity normally attributed to starvation was minimized (35). The administration of SAD also delayed the increase in free fatty acids and β-hydroxybutyrate concentrations occurring during starvation.

It was speculated, therefore, that SAD could be used to prevent the imbalance between ATP generation and breakdown in selected metabolic situations. Supporting evidence was obtained in a study in which the infusion of 80 μmol/g/day of SAD was found to protect rats against the deleterious effects of 5 μg/g of bacterial lipopolysaccharide (LPS) on such variables as the plasma concentration of free fatty acids and β-hydroxybutyrate, liver ATP content and oxidation of D-glucose, as well as the pyruvate/lactate ratio in hepatocytes prepared from LPS-injected rats (36). The administration of SAD also suppressed lethality in LPS-injected animals. Thus, succinic acid esters could represent a novel therapeutic approach for endotoxemia and multiple organ failure.

The research on SAD hepatic metabolism was recently extended to experiments conducted in hepatocytes from normal or starved rats and GK rats incubated in the presence of the dimethyl ester of [2,3-^{13}C]succinic acid (37-39). These experiments led to the identification and quantification of several ^{13}C-labeled metabolites of the ester such as succinate, fumarate, malate, lactate, alanine and glucose, as well as documenting the influence of starvation and diabetes.

Several approaches were used to assess the possible long-term effects of SAM and SAD on the endocrine pancreas of normal and diabetic rats. It was first shown in islets from control and GK rats cultured for 3 days in the absence or presence of SAM (10 mM) that the ester had no effect on the activity of FAD-linked glycerophosphate dehydrogenase and oxidation of D-[U-^{14}C]glucose (3.3 and 16.7 mM). There was no difference in the rate of insulin release recorded over 60 min incubation at low (3.3 mM) and high (16.7 mM) D-glucose concentrations from the islets of normal rats cultured in the presence and absence of SAM (paired ratio = 115.7 ± 12.8%). However, islets from GK rats, which exhibited a lower rate of glucose-stimulated insulin release, had a slightly higher output of insulin after incubation in the presence, rather than absence, of SAM (paired ratio = 143.7 ± 15.6%) (40).

Repeated pulse administration of SAM (0.45 mmol or ca. 2.0 μmol/g i.p. t.i.d. for 1 week) to adult rats 5 days after a high dose of STZ (40 μg/g) was found to improve the secretory response of the isolated perfused pancreas

to SAM in the presence of 6.0 mM D-glucose; no effect was observed in animals injected with a low dose of STZ (20 μg/g) (41). Comparable results were obtained in pancreatic islets prepared from STZ rats incubated in the absence or presence of 2-ketoisocaproate (10 mM) or the combination of D-glucose (6.0 mM) and SAM (10.0 mM) (42).

Because the intragastric administration of SAD (10 μmol/g) resulted in both the appearance of the ester in blood and a marked increase in plasma insulin concentration (20, 21), an attempt was made to incorporate the ester (0.67 M) in the drinking water given to rats for 6 days (43). However, the SAD solution had an unpleasant smell and animals drank less than 0.6 ml/day. In addition, they did not eat, they lost weight, had bristly hair, were quiet and remained huddled together. After 6 days, there was a marked decrease in pancreatic weight and insulin content, but not glucagon content. Consequently, a procedure for conditioning normal rats to oral administration of SAD was developed (44). Animals had access to a drinking solution containing SAD in increasing concentrations together with an artificial sweetener. Over a period of 8 weeks, the rats eventually were able to drink about 10 ml/day of a 0.54 M solution of SAD, with a food intake maintained at 10.8 ± 0.3 g/day. Nevertheless, when the SAD-conditioned rats were starved for 3 days and given free access to either tap water or a saturated solution of SAD (0.67 M), the ester failed to prevent the decrease in body weight, plasma D-glucose and insulin concentration, liver glucokinase activity, and pancreatic islet secretory responsiveness to D-glucose, as well as the increase in plasma free fatty acids and β-hydroxybutyrate concentration normally attributed to starvation (45). It was concluded that the rate of SAD absorption was too low to compensate for the effect of starvation upon the pancreatic B cell secretory potential.

Studies using 3 experimental models demonstrated the long-term effects of SAM on preventing the impairment of glucose-stimulated insulin secretion caused by hexose deprivation or starvation (46). In the first model, preincubation of the islets for 180 min at a low glucose concentration in the presence of SAM prevented the decrease in the secretory response to D-glucose normally observed during a subsequent incubation. In the second model, an impaired secretory response to D-glucose was observed after a 3-day culture at a low (2.8 or 5.6 mM) compared to high (11.1 mM) hexose concentration and the presence of SAM in the culture medium again protected against this anomaly. In the third model, the infusion of SAM for 3 days to starved rats restored the secretory potential of isolated islets to a level comparable to that observed in fed rats. Thus, SAM maintained B cell responsiveness to D-glucose during glucose deprivation and starvation.

Similarly, the administration of SAD (80 μmol/g/day) for 3 days to starved rats prevented the decreases in plasma D-glucose and insulin concentrations and the impairment of glucose-induced insulin release from isolated islets caused by starvation (47).

The possible metabolic determinants of such a protective action were then examined (48). After 180 min preincubation of islets with 2.8 mM D-glucose in the presence of SAM (10 mM), the oxidation of D-[U-^{14}C]glucose, relative to either the utilization of D-[5-^{3}H]glucose or the generation of ^{14}C-labeled acidic metabolites, was higher as compared to islets preincubated in the absence of SAM; the value obtained was close to that observed after preincubation with 16.7 mM D-glucose. Similarly, after 3 days culture at a low concentration of D-glucose (2.8 mM), the presence of SAM in the culture medium tended to increase the subsequent oxidation of D-[6-^{14}C]glucose and utilization of D-[5-^{3}H]glucose. These two variables increased as a function of the concentration of D-glucose in the culture medium and this coincided with a modest increase in hexokinase activity and a more pronounced increase in glucokinase activity. However, in the presence of SAM in the culture medium, no obvious effect on respiration of the islets was observed, suggesting that the protective action of the ester against glucopenia may also involve variables distinct from the metabolism of either endogenous or exogenous nutrients. Likewise, the fact that SAM prevented the impairment of glucose-induced insulin release in starved rats may be due to enzymatic determinants such as a less severe decrease in glucokinase activity, metabolic variables including a greater increase in D-[U-^{14}C]glucose oxidation relative to D-[5-^{3}H]glucose utilization in response to a rise in extracellular D-glucose concentration, and to other as yet unidentified factors involved in the secretory sequence at a site distal to those metabolic events triggered by D-glucose in the islet cells.

In order to explore the possible long-term effects of SAM in diabetic rats, either saline or SAM (36-47 μmol/g/day) was infused for 3 days to control, STZ and GK rats (49). SAM failed to correct the anomalies found in the islets of diabetic rats, namely, decreased activity of the mitochondrial FAD-linked glycerophosphate dehydrogenase, low insulin content and impaired secretory response to various nutrient secretagogues including D-glucose, 2-ketoisocaproate and the combination of L-leucine and L-glutamine. These findings raise the question of whether a more prolonged administration of SAM is required to raise the insulin store and improve the secretory potential of the endocrine pancreas in animals with type-2 diabetes.

Monosaccharide esters of succinic acid

To avoid the undesirable generation of methanol that could result from *in vivo* administration of SAM or SAD, studies were performed to investigate whether the esterification of the carboxyl group of succinic acid monomethyl ester by D-glucose or 3-*O*-methyl-D-glucose affected its insulinotropic action (50). Both the 6-*O*-D-glucosyl and 6-*O*-(3-*O*-methyl)-D-glucosyl esters were found to stimulate insulin secretion in pancreatic islets (Table I) and the isolated perfused pancreas. The 6-*O*-D-glucosyl ester

Table I: Insulinotropic effects of the 6-O-succinyl esters of hexoses and related molecules.

Ester Nr.	Name	Insulinotropic Action *In vitro* Concentration	*In vitro* Effect	*In vivo*[c] Dose	*In vivo*[c] Effect	Ref.
1	6-*O*-(Methylsuccinyl)-D-glucose	10.0 mM	≅1.5 mM D-glucose[a]	9.9 μmol/g	≤3.1 pmol/g D-glucose	50
2	6-*O*-(Methylsuccinyl)-3-*O*-methyl-D-glucose	10.0 mM	≤ Nr-1[a]	N.D.		50
3	6-*O*-(Isopropylsuccinyl-D-galactose	10.0 mM	No significant effect[a]	N.D.		51
4	6-*O*-(Hydrogensuccinyl)-D-galactose	10.0 mM	Modest effect (<Nr.5)[a]	N.D.		51
5	6-*O*-(Isopropylsuccinyl)-3-*O*-methyl-D-glucose	10.0 mM	≅1.0 mM D-glucose[a]	2.0 μmol/g	≤Nr. 6	51,52
6	6-*O*-(Methylsuccinyl)-D-galactose	10.0 mM	≥Nr. 5[a]	2.0 μmol/g	≥Nr. 1	51,52
7	6-*O*-(Hydrogensuccinyl)-3-*O*-methyl-D-glucose	10.0 mM	No effect[b]	N.D.		53
8	6-Amino-(methylsuccinyl)-6-deoxy-3-*O*-methyl-D-glucose	10.0 mM	<<Nr. 5[b]	N.D.		53
9	6-Amino-(methylsuccinyl)-6-deoxy-D-galactose	10.0 mM	<<Nr. 5[b]	N.D.		53

[a]At 6-0 mM D-glucose. [b]At 7.0 mM D-glucose. [c]Intravenous injection in anesthetized fed rats. N.D.: Not determined.

also stimulated insulin release after intravenous administration to anesthetized rats (Table I). These findings indicate that the production of methanol from the methyl esters of succinic acid could eventually be avoided by using other esters of this dicarboxylic acid, while maintaining the benefit of their insulinotropic action.

Results from further work conducted along the same line indicated that 6-*O*-isopropylsuccinyl-α-D-galactopyranose, 6-*O*-hydrogensuccinyl-α-D-galactopyranose, 6-*O*-isopropylsuccinyl-3-*O*-methyl-α-D-glucopyranose and 6-*O*-methylsuccinyl-α-D-galactopyranose in order of increasing potency augmented the release of insulin by rat islets incubated in the presence of 6.0-7.0 mM D-glucose (51) (Table I). The intravenous administration of 6-*O*-methylsuccinyl-α-D-galactopyranose and, to a lesser extent, that of 6-*O*-isopropylsuccinyl-3-*O*-methyl-α-D-glucopyranose (each 2.0 μmol/g), also caused an increase in plasma insulin concentration within 2 min in anesthetized fed rats (52). Thus, suitable esters of succinic acid which do not generate an undesirable alcohol by intracellular hydrolysis, may indeed be appropriate tools for stimulating the endocrine pancreas in long-term experiments conducted *in vivo*. Such is the case even when the monosaccharide moiety of these esters corresponds to a noninsulinotropic sugar (*e.g.*, D-galactose or 3-*O*-methyl-D-glucose). However, 6-*O*- hydrogensuccinyl-3-*O*-methyl-D-glucose ester, 6-amino-(methylsuccinyl)-6-deoxy-3-*O*-methyl-D-glucose and 6-amino-(methylsuccinyl)-6-deoxy-D-galactose were rather poor secretagogues (53).

Novel esters of succinic acid

More recently, a series of novel esters of succinic acid were examined for their insulinotropic action.

As indicated in Table II, the monoethyl ester of succinic acid (10.0 mM) exerted an insulinotropic effect in rat pancreatic islets comparable to that of 5.2 mM D-glucose (54). This ester was efficiently metabolized, as judged from the generation of $^{14}CO_2$ by islets exposed to the monoethyl ester of either [1,4-^{14}C] or [2,3-^{14}C]succinic acid. This ester also stimulated biosynthetic activity in islets exposed to 4.2 mM D-glucose and inhibited ^{86}Rb and ^{45}Ca outflow from prelabeled islets perifused in the absence of D-glucose while enhancing the efflux of the two cationic tracers in the presence of the hexose (7 mM). It was concluded that the insulinotropic action of this ester could largely be attributed to its capacity to act as a nutrient in islet cells.

Succinic acid monoethyl ester also stimulated insulin secretion *in vivo* (see below). Its nutritional value was further documented by administration of the sodium salt of monoethyl succinic acid (80 μmol/g) to food-deprived rats for 48 h (55). In comparison to starved rats given an equimolar amount of NaCl, the succinate ester increased plasma D-glucose concentration, delayed the rise in ketonemia, maintained a higher glucokinase/hexokinase activity ratio in liver and pancreatic islets, and allowed for more efficient stimulation of insulin release by D-glucose in pancreatic islets.

When compared to the monoethyl ester of succinic acid, the monopropyl, monoisopropyl, monoethyl and monobenzyl esters were less potent secretagogues (Table II). However, the monobutyl ester was as potent as the monoethyl ester, while the monohexyl ester was apparently devoid of insulinotropic action (53). The 4-*tert*-butyl hydrogen ester of succinic acid also stimulated insulin secretion in islets exposed to 2.8-7.0 mM D-glucose, with a maximal secretory response observed with an ester concentration of 5.0 mM (56).

Similarly, the dipropyl, diallyl and dibutyl esters of succinic acid (10 mM) significantly enhanced insulin release evoked by 7.0 mM D-glucose in rat pancreatic islets (53). This was not the case, however, with the diethyl, diisopropyl, disalicyl and dimenthyl esters of succinic acid (Table II).

Within 2 min after administration of the monoethyl, monopropyl, monoisoproyl, monoallyl and diallyl esters of succinic acid (2 μmol/g i.v.) to anesthetized rats, plasma insulin concentrations increased (57). On a molar basis, however, these esters appeared to be less potent insulin secretagogues than the methyl esters of succinic acid. The monoethyl, monopropyl and monoisopropyl esters of succinic acid also enhanced the insulinotropic action of gliquidone in anesthetized fed rats (58). These findings

Table II: Insulinotropic effects of novel esters of succinic acid.

Ester Nr.	Name	Insulinotropic Action *In vitro* Concentration	*In vitro* Effect[a]	*In vivo*[b] Dose	*In vivo*[b] Effect	Ref.
1	Ethyl hydrogen succinic acid	10.0 mM	≅5.2 mM glucose[a]	2.0 μmol/g	≥1.1 μmol/g D-glucose	53,57
2	Propyl hydrogen succinic acid	10.0 mM	≤ Nr. 1	2.0 μmol/g	> Nr. 1	53,57
3	Isopropyl hydrogen succinic acid	10.0 mM	≤ Nr. 2	2.0 μmol/g	≅ Nr. 1	53,57
4	Allyl hydrogen succinic acid	10.0 mM	≤ Nr. 2	2.0 μmol/g	≅ half of Nr. 1	53,57
5	Butyl hydrogen succinic acid	10.0 mM	≅ Nr. 1	N.D.	N.D.	53
6	Hexyl hydrogen succinic acid	10.0 mM	No effect	N.D.	N.D.	53
7	Benzyl hydrogen succinic acid	10.0 mM	≤ Nr. 3	N.D.	N.D.	53
8	Diethyl succinic acid	10.0 mM	No effect	N.D.	N.D.	53
9	Dipropyl succinic acid	10.0 mM	≅ Nr. 3	N.D.	N.D.	53
10	Diisopropyl succinic acid	10.0 mM	No effect	N.D.	N.D.	53
11	Diallyl succinic acid	10.0 mM	≅ Nr. 9	2.0 μmol/g	≤ Nr. 5	53,57
12	Dibutyl succinic acid	10.0 mM	≤ Nr. 9	N.D.	N.D.	53
13	Disalicyl succinic acid	10.0 mM	No effect	N.D.	N.D.	53
14	Dimenthyl succinic acid	10.0 mM	No effect	N.D.	N.D.	53

[a]Tested at 7.0 mM D-glucose. [b]Intravenous injection in anesthetized fed rats. N.D.: Not determined.

indicate that it is possible to design esters of succinic acid that do not generate methanol via intracellular hydrolysis, and yet are still capable of increasing the insulinotropic response to classical antidiabetic agents.

Polyol esters of succinic acid

In our most recent work on succinic acid esters, attention was focused on several polyol esters of the dicarboxylic acid. In this case, each molecule of the ester contains 2-4 succinyl residues. The pilot and most extensive study on such polyol esters concerns glycerol-1,2,3-tris(methylsuccinate) or 3SMG. It was first shown that a 10 μM concentration of 3SMG was sufficient to cause a significant increase in insulin output from islets incubated in the presence of 7 mM D-glucose (59). The ester mimicked the effect of other nutrient secretagogues in enhancing the synthesis of islet peptides, with preferential action on proinsulin as opposed to nonhormonal peptides, and decreased ^{86}Rb efflux while stimulating Ca^{2+} influx in prelabeled islets. Moreover, 3SMG (0.01-0.2 mM) dose-dependently stimulated the formation of tritiated inositol phosphate in islets prelabeled with myo-[2-^{3}H]inositol and subsequently incubated for 30 min with 7 mM D-glucose in the presence of 10 mM LiCl to prevent inositol phosphate degradation (60). Lastly, the insulinotropic effect of the hexose was augmented when islets were perifused for 15 min with 3SMG (0.05-0.2 mM) together with 7.0 mM D-glucose. Under these experimental conditions, 3SMG also caused a time-dependent potentiation of the early secretory response of the islets to a second hexose exposure (15.0 mM) introduced following a 15 min perifusion of D-glucose (3 mM) (60). It was proposed, therefore, that 3SMG displays the attributes necessary for stimulating or potentiating insulin release in noninsulin-dependent diabetes, without requiring administration of large amounts and, hence, avoiding the risk of excessive hepatic gluconeogenesis.

The metabolism of 3SMG (2.0 mM) was then examined in rat pancreatic islets (61). The oxidation of the glycerol moiety of the ester was negligible compared to that of its succinate residues. The oxidation of glycerol-1,2,3-tris(methyl-[1,4-^{14}C] succinate) was 2-fold higher than that of glycerol-1,2,3-tris(methyl-[2,3-^{14}C]succinate). This difference was matched by a higher generation of ^{14}C-labeled acidic metabolites and amino acids from the latter than from the former tracer. The total generation of $^{14}CO_2$ from the ester, uniformly labeled except in its methyl groups, was similar to that found for the oxidation of 1.0 mM D-[U-^{14}C]glucose. These findings demonstrated that 3SMG is efficiently metabolized in islet cells, suggesting that this ester could be used as a nutrient to bypass defects in D-glucose transport and metabolism in B cells, thus resulting in improved proinsulin biosynthesis and insulin release in noninsulin-dependent diabetes mellitus.

In experiments conducted in anesthetized fed rats, as little as 70 nmol/g i.v. of 3SMG was found to stimulate insulin release and potentiate the insulinotropic action of gliquidone (0.2 nmol/g) and repaglinide (0.1 nmol/g) (62). These findings indicate that novel succinic acid esters with high insulinotropic potency overcome two of the major limitations associated with this new therapeutic approach to noninsulin-dependent diabetes; namely, avoiding the need for large amounts of a drug to achieve a sizeable stimulation of insulin release and reducing the risk of excessive gluconeogenesis (1, 29).

Moreover, 3SMG (0.03-1.0 mM) caused a concentration-related stimulation of insulin release evoked by 7.0 mM D-glucose in islets from GK rats (63). At this hexose concentration, as little as 0.1 mM 3SMG was sufficient to augment insulin secretion to a rate similar to that achieved with a near maximal concentration of D-glucose (16.7 mM). Similarly, 3SMG stimulated the incorporation of L-[4-^{3}H]phenylalanine into TCA-precipitable material in the islets from the diabetic animals. Even in the absence of any other exogenous nutrient, 3SMG (2.5 mM)

increased biosynthetic activity above base values, to a level not lower than that recorded in the presence of 16.7 mM D-glucose alone. These results show that 3SMG is very efficient as an insulinotropic agent in this animal model of type-2 diabetes.

Studies have also shown that 3SMG is efficiently metabolized in hepatocytes. The output of $^{14}CO_2$ from rat hepatocytes exposed to 1.9 mM [U-^{14}C]glycerol-1,2,3-tris(methylsuccinate), glycerol-1,2,3,-tris(methyl[1,4-^{14}C]-succinate) or glycerol-1,2,3-tris(methyl[2,3-^{14}C]succinate) yielded an approximately 50-fold higher value than that found in cells incubated with 1.0 mM D-[U-^{14}C]glucose. For glycogen synthesis, the values obtained with the ester were 7- to 8-fold higher than those found with the hexose (64). Complementary information on the metabolic fate of 3SMG in hepatocytes was obtained in experiments conducted in the presence of [1,3-^{13}C]glycerol-1,2,3-tris(methylsuccinate) or glycerol-1,2,3-tris-(methyl[2,3-^{13}C]succinate), in which case the production of double- or single-labeled succinate, fumarate, malate, lactate and alanine, as well as the various ^{13}C-labeled isomers of D-glucose, could be measured (65). In addition, 3SMG (2.0 mM) incorporation into the Belzer UW-CSS solution used for liver perfusion and storage for 20 h at 4 ºC restored the viability of the hepatocytes to near normal values and prevented the starvation-like effects of liver storage on both the conversion of D-[U-^{14}C]glucose to $^{14}CO_2$ and radioactive amino acids and the *de novo* generation of ^{14}C-labeled D-glucose from [2-^{14}C]pyruvate (66). Because succinic acid esters are efficiently metabolized in several cell types, these results suggest that such esters may have a wide application in transplantation procedures.

Our experimental work with 3SMG was extended to 10 other polyol esters of succinic acid. Glycerol-1,2,3-tris-(ethylsuccinate) was first found to exert a dual effect on insulin release (67). It augmented insulin output when tested in the 0.1-0.5 mM range in islets incubated in the presence of 7.0 mM D-glucose, but inhibited hormonal secretion when used either in a higher concentration (1.0 mM) or in the presence of a higher concentration of D-glucose (16.7 mM). Comparable findings were obtained with ethanediol-1,2-dimethylsuccinate, ethanediol-1,2-diethylsuccinate, propanediol-1,2-dimethylsuccinate, glycerol-1,2-dimethylsuccinate, glycerol-3-hydroxy-1,2-dimethylsuccinate, glycerol-1,2-dimethylsuccinate-3-hydrogenosuccinate, L-threitol-1,2,4-trimethylsuccinate, D-arabitol-5-hydroxy-1,2,3,4-tetramethylsuccinate, and L-threitol-3-succinoyl-1,2,4-trimethylsuccinate (56). The minimal effective concentration of these esters ranged between 10 μM and 2.5 mM. There was a close correlation between the minimal effective concentration and the insulinotropic potential of the esters as determined from criteria such as the rate of insulin release at a given concentration of each ester in the presence of a given concentration of D-glucose. In the presence of these esters, the concentration-response relationship for glucose-stimulated insulin release changed from a typical sigmoidal shape to a hyperbolic pattern (56). There was also a significant correlation between the insulinotropic action of these esters and their capacity to stimulate biosynthetic activity in islets deprived of any other exogenous nutrient (68).

Two of the latter esters, namely propanediol-1,2-dimethylsuccinate (1.0 mM) and glycerol-1,2-dimethylsuccinate (2.5 mM), were also examined for their effects upon insulin release and protein biosynthesis in islets from GK rats (69). Although these two esters only modestly increased insulin release at low (2.8 mM), intermediate (7.0 mM) or high (16.7 mM) D-glucose concentrations, both markedly increased the incorporation of L-[4-^{3}H]phenylalanine into TCA-precipitable material in islets deprived of any other exogenous nutrient. These findings again indicate that, in contrast to all compounds presently in use or proposed as insulin secretagogues in the treatment of type-2 diabetes, propanediol-1,2-dimethylsuccinate and glycerol-1,2-dimethylsuccinate, considered as islet cell nutrients with insulinotropic action, also stimulate biosynthetic activity in the endocrine pancreas of animals with type-2 diabetes.

Glutamic dimethyl ester

Glutamic acid, like succinic acid, does not efficiently penetrate pancreatic islets and has virtually no insulinotropic capacity. However, the dimethyl ester of glutamic acid (GME) efficiently stimulated insulin release from B cells in a manner comparable to that previously reported for islets exposed to L-glutamine (70). It was first shown that GME (3.0-10.0 mM) enhanced insulin release evoked by D-glucose (6.0-8.3 mM), L-leucine (1.0-10.0 mM) or 2-amino-bicyclo(2,2,1)heptane-2-carboxylic acid (5.0-10.0 mM) in rat pancreatic islets (70). GME caused a shift to the left of the sigmoidal relationship between insulin output and D-glucose concentration. In the absence of D-glucose, GME also unmasked the insulinotropic potential of glibenclamide (70) and several meglitinide analogs (71). In islets exposed to L-leucine, the insulinotropic action of GME coincided with an early fall and later increase in ^{86}Rb efflux and augmentation of ^{45}Ca outflow from prelabeled islets. The measurement of O_2 uptake, NH_4^+ output and content (72), the production of $^{14}CO_2$ from islets prelabeled with [U-^{14}C]palmitate, the generation of ^{14}C-labeled amino acids and $^{14}CO_2$ from the dimethyl ester of either L-[1-^{14}C]glutamic acid or L-[U-^{14}C]glutamic acid, and D-[2-^{14}C]glucose as well as D-[6-^{14}C]glucose oxidation in the presence or absence of GME indicated that the latter ester was efficiently converted to L-glutamate and its further metabolites. The overall gain in O_2 uptake represented the balance between GME oxidation and its sparing action on the catabolism of endogenous fatty acids and exogenous D-glucose. It was proposed that GME might represent a new tool to bypass B-cell defects in D-glucose transport, phosphorylation and further metabolism, thus stimulating insulin release in animal models of noninsulin-dependent diabetes mellitus (70). In the isolated rat pancreas

perfused in the presence of 6.0 mM D-glucose, the administration of 10 mM GME caused a rapid and sustained increase in both insulin and glucagon output (70).

Moreover, in a reverse hemolytic plaque assay for insulin release, GME (3 mM) significantly increased the percentage of plaque-forming cells and the insulin secretion index, taken as the product of mean plaque area by the percentage of plaque-forming cells, from individual B cells exposed to 5 mM L-leucine (7).

Although the enteral or intraduodenal administration of GME (2.5-10 μmol/g) to overnight fasted rats only caused a minor increase in plasma insulin concentration (21, 22), within 2 min after intravenous injection of the ester (<1.0 μmol/g) to fed rats there was about a 4-fold increase in plasma insulin concentration (73). In overnight fasted and anesthetized rats, GME (1.0 μmol/g i.v.) increased plasma insulin concentrations within 2 min by 1.07 ± 0.33 ng/ml above base values (74). In fasted, but not fed, rats GME potentiated the secretory response to glibenclamide (0.1 nmol/g).

Intravenous administration of GME and/or glibenclamide also significantly increased plasma insulin concentrations in adult STZ rats (74). In this animal model of type-2 diabetes, like in GK rats, the oxidation of the dimethyl ester of L-[U-^{14}C]glutamic acid (3.0 mM) by isolated islets was not different from that found in control rats provided that the results are expressed relative to the amount of unesterified ^{14}C-labeled amino acids (mainly L-[U-^{14}C]glutamate) available for oxidation (13).

In addition, GME was also found to be efficiently metabolized in non-islet cells, such as colon carcinoma cells (33).

Pyruvic acid methyl ester

Pyruvate, although efficiently metabolized in pancreatic islets, is a relatively poor insulinotropic nutrient. This is mainly due to the consumption of NADH, associated with the intracellular conversion of pyruvate to L-lactate, and to a pronounced sparing action of the 2-keto acid on the oxidation of endogenous fatty acids in islet cells.

Recent studies have investigated the possibility of enhancing the insulinotropic action of this 2-keto acid by using methyl pyruvate instead of pyruvate. Methyl pyruvate was found to exert a dual effect on insulin release from isolated rat pancreatic islets. A positive insulinotropic action was evident at low concentrations of D-glucose (2.8-8.3 mM) and at concentrations of the ester <10.0 mM. The ester displayed features typical of nutrient-stimulated insulin release such as decreased K^+ conductance, enhanced Ca^{2+} influx and stimulation of proinsulin biosynthesis. However, a negative insulinotropic action of methyl pyruvate was also observed at high concentrations of D-glucose (16.7 mM) and/or the methyl ester (20.0 mM), which may have been due to hyperpolarization of the plasma membrane rather than adverse effects of methyl pyruvate on ATP generation. The ionic determinant(s) of this hyperpolarization was not identified. The dual effects of methyl pyruvate probably account for an unusual time course of the secretory response, including a dramatic and paradoxical stimulation of insulin release upon removal of the ester (75). In the absence of exogenous nutrients, methyl pyruvate (10 mM) does not affect basal insulin output and fails to support the insulinotropic action of meglitinide analogs (71).

The metabolism of methyl pyruvate was then compared to that of pyruvate in isolated rat pancreatic islets. Methyl pyruvate was found to be more efficient than pyruvate in supporting the intramitochondrial conversion of pyruvate metabolites to amino acids, inhibiting D-[5-^{3}H]glucose utilization, maintaining a high ratio between D-[3,4-^{14}C]glucose or D-[6-^{14}C]glucose oxidation and D-[5-^{3}H]glucose utilization, inhibiting the intramitochondrial conversion of glucose-derived 2-keto acids to their corresponding amino acids, and increasing $^{14}CO_2$ output from islets prelabeled with L-[U-^{14}C]glutamine. Methyl pyruvate also appeared to cause a more marked mitochondrial alkalinization than pyruvate, as judged from comparisons of pH measurements based on the use of either a fluorescein probe or ^{14}C-labeled 5,5-dimethyl-oxazolidine-2,4-dione. Inversely, pyruvate was more efficient than methyl pyruvate in increasing lactate output and generating L-alanine. These findings indicate that, in comparison to exogenous pyruvate, its methyl ester is preferentially metabolized in the mitochondrial rather than the cytosolic domain of islet cells. It has been proposed that both the positive and negative aspects of methyl pyruvate's insulinotropic action are related to changes in the net generation of reducing equivalents, ATP and H^+ (76).

In contrast to the results obtained in perifused islets, exposure of the isolated perfused rat pancreas to methyl pyruvate (10 mM) resulted in a rapid and sustained increase of insulin release evoked by either 7.0 or 16.7 mM D-glucose. Under these conditions, methyl pyruvate caused a modest, biphasic stimulation of glucagon release (77).

In anesthetized fed rats, methyl pyruvate (1.0-2.5 μmol/g i.v.) produced a short-lasting and dose-related increase in plasma insulin concentration, but failed to affect plasma glucagon concentration. When administered together, D-glucose and methyl pyruvate had an additive effect on insulin release. The *in vivo* secretory response to methyl pyruvate was comparable in fed, overnight fasted and 2-day starved rats, and was only slightly decreased in fed STZ animals. These results suggest that methyl pyruvate could be used as an insulinotropic agent to prevent site-specific defects of D-glucose metabolism in the B cells, such as those found in starvation or noninsulin-dependent diabetes mellitus (77).

Acetic acid esters

At increasing concentrations of D-glucose (0, 1.7, 8.3 and 16.7 mM) or in the presence of 10.0 mM L-leucine, acetic acid (8.5-10.0 mM) and its methyl or ethyl ester

(9.4-10.2 mM) produced only minor changes in insulin output from rat pancreatic islets (78). However, the methyl ester of acetic acid (10.0 mM), but not its ethyl ester (10.0 mM), significantly increased insulin release in the presence of 10.0 mM succinic acid dimethyl ester in islets from both normal and GK rats (79). These findings may be explained by the existence of a threshold value for stimulation of insulin release at increasing rates of O_2 consumption and by a reciprocal action of acetic acid or its esters and other nutrients on their respective catabolism in the islet cells (79).

Monosaccharide esters

In recent years, the use of nutrient esters has been extended to esters of several monosaccharides as a possible method for improving their passage across the B-cell plasma membrane. For example, α-D-glucose pentaacetate penetrated efficiently into islet cells, apparently without the need for a specific carrier system (80). The ester underwent intracellular hydrolysis in esterase-catalyzed reactions so that large amounts of D-glucose became available for phosphorylation and further metabolism. As a matter of fact, α-D-glucose pentaacetate is more efficiently metabolized and stimulates insulin secretion to a greater extent than unesterified D-glucose tested at the same molar concentrations (78, 81).

Likewise, D-mannoheptulose hexaacetate inhibited D-glucose metabolism in cells resistant to the unesterified heptose (82). The tetraacetate of 2-deoxy-D-glucose was also more potent than unesterified 2-deoxy-D-glucose in inhibiting D-glucose metabolism in pancreatic islets and erythrocytes (83), and as a cytostatic and/or cytotoxic agent in several tumor cell lines (84-87). Since the use of monosaccharide esters as new tools in biomedical research was recently reviewed (88), only three further points will be discussed here.

First, since it is known that, in contrast to succinic acid itself, several of its esters are potent stimulators of insulin release and selected esters of D-glucose stimulate insulin release more efficiently than the unesterified hexose, we considered whether esters formed of both D-glucose and succinic acid could conceivably display more potent secretory activity (89). D-Glucose-1,2,3,4,6-pentamethylsuccinate (1.0 mM) markedly enhanced insulin release evoked by either D-glucose (6.0 mM) or L-leucine (10.0 mM). However, this did not occur with D-glucose-1,2,3,4-6-pentaethylsuccinate, D-glucose-1,2,3,4,6-pentaisopropylsuccinate or D-glucose-1,2,3,4,6-pentabutylsuccinate.

Second, the combined effects of succinic acid monomethyl or dimethyl ester and selected monosaccharide esters on insulin release were examined in islets from both normal and GK rats. In one study using islets from normal rats, the increase in the secretory rate attributed to α-D-glucose pentaacetate (1.7 mM) was higher in the presence of succinic acid monomethyl ester (10.0 mM) than in its absence (79). Likewise, in islets from GK rats, the combination of α-D-glucose pentaacetate and succinic acid monomethyl ester significantly increased insulin release above base values, an effect not observed when the esters were tested alone (79). With the exception of β-L-glucose pentaacetate, the relative enhancing effect of monosaccharide esters such as β-D-glucose penta-acetate and D-mannoheptulose hexaacetate (1.7 mM) on insulin release evoked by the dimethyl ester of succinic acid (10.0 mM) was even higher in islets from GK rats compared to those of control animals (90).

Finally, although most monosaccharide esters (*e.g.*, α-D-glucose pentaacetate, β-D-glucose pentaacetate, α-L-glucose pentaacetate, β-L-glucose pentaacetate, 2-deoxy-α-D-glucose pentaacetate, 2-deoxy-β-D-glucose pentaacetate and D-mannoheptulose hexaacetate) increased insulin release evoked by succinic acid dimethyl ester (90-93), α-D-galactose pentaacetate, but not β-D-galactose pentaacetate (1.7 mM each), was recently found to inhibit the secretory response to the succinic acid ester (92, 93). Hence, it was proposed that the use of α-D-galactose pentaacetate would be advantageous in preventing excessive insulin secretion in conditions such as persistent hyperinsulinemia in childhood or insulinoma.

Conclusions

The information in this review demonstrates that the introduction of nutrient esters, first considered as new insulinotropic agents for the treatment of noninsulin-dependent diabetes mellitus, eventually led to their potential use not only in diabetes but also in other pathological conditions such as endotoxemia, multiple organ failure, persistent hyperinsulinemia in childhood and in organ transplantation. Further work is presently in progress to extend the use of these nutrient esters to other indications, including strenuous muscular exercise and brain glucopenia.

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