Glyceraldehyde Phosphate and Methyl Esters of Succinic Acid

Two "New" Potent Insulin Secretagogues

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We discovered that two physiologically occurring metabolic intermediates, glyceraldehyde phosphate and succinate, are potent insulin secretagogues. No other glycolytic intermediate besides glyceraldehyde phosphate was insulinotropic. Succinate, when added to islets as either its monomethyl or dimethyl ester to increase its cellular permeability, was also insulinotropic. In islets, as in other cell types, these esters are apparently hydrolyzed intracellulary to succinate. Unesterified succinate and other unesterified citric acid–cycle intermediates did not stimulate insulin release. Initiation of insulin release by esters of succinate suggests that mitochondrial metabolism alone is sufficient to initiate and support insulin release. However, this is specific for succinate in that esters of fumarate, pyruvate, and citrate were not insulinotropic. *Diabetes* 37:997–99, 1988

The pancreatic β-cell may be unique because the primary physiologic stimulus for cellular excitation, i.e., insulin release, involves the metabolism of the secretagogue rather than only the interaction of the secretagogue with a receptor. Although numerous agents have been used to study insulin release, there are no reports of intermediary metabolites stimulating insulin release. We report, in preliminary form, the results of extensive testing of intermediary metabolites for their ability to stimulate insulin release from isolated pancreatic islets. Two compounds proved to be insulin secretagogues—glyceraldehyde phosphate and esters of succinate.

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MATERIALS AND METHODS

Standard procedures were used to isolate pancreatic islets from well-fed Sprague-Dawley rats (1–3), to incubate islets for studying insulin release (5 islets in 2 ml Krebs-Ringer bicarbonate buffer, pH 7.35, containing 0.5% bovine serum albumin for 1 h; 3,4), and to assay insulin (5). Succinic acid monomethyl and monoethyl esters and fumaric acid monoethyl and dimethyl esters were from Pfaltz and Bauer (Waterbury, CT). D-Glucose was from the National Bureau of Standards (Washington, DC). All other chemicals, in the highest purity available, were from Sigma (St. Louis, MO). Three different sources of glyceraldehyde 3-phosphate were used interchangeably—the free acid (an equal mixture of the D- and L-isomers and two types that were prepared by hydrolysis of the diethyl diacetal salt (the D-isomer only and an equal mixture of the D- and L-isomers) in the presence of Dowex 50 resin as described by the supplier. Concentrations of glyceraldehyde phosphate refer to the physiologically active D-isomer determined with enzymatic measurements (6). Student's *t* test was used to estimate statistical significance.

RESULTS AND DISCUSSION

Table 1 shows typical results of insulin-release experiments with glyceraldehyde phosphate and methyl esters of succinate and compares the results with those of the well-known secretagogues glucose, leucine, and glyceraldehyde. Glyceraldehyde 3-phosphate was the only glucose metabolite tested that proved to be a potent insulin secretagogue. At concentrations of 1–4 mM, glyceraldehyde phosphate was a more potent secretagogue than comparable concentrations of glyceraldehyde and was more potent than 5.5 mM glucose. Dihydroxyacetone phosphate and dihydroxyacetone at concentrations of 4–20 mM usually exhibited weak secretagogue activity. Glycolytic intermediates that failed to elicit insulin release when tested at a concentration of 10 mM were glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, and pyruvate. Glycerol 3-phosphate, 2,3-diphosphoglycerate, ribose 5-phosphate,

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TABLE 1
Insulin release from pancreatic islets in presence of glyceraldehyde phosphate, methyl esters of succinate, and other secretagogues

Addition (mM)	Insulin release ($\mu U \cdot 5$ islets$^{-1} \cdot h^{-1}$)	*n*
None	16 ± 8	48
Glucose		
5.5	55 ± 13	15*
16.7	262 ± 37	18*†
Leucine, 10	108 ± 23	15*†
Glyceraldehyde phosphate		
0.5	42 ± 9	7*
1	55 ± 20	17*‡
2	79 ± 31	8*
4	139 ± 26	24*†
8	195 ± 37	10*†
Dihydroxyacetone phosphate		
4	32 ± 11	8*
20	57 ± 12	8*
Glyceraldehyde		
1	29 ± 7	12
4	68 ± 12	18*
10	181 ± 23	3
20	235 ± 21	5
Dihydroxyacetone		
4	22 ± 12	4
20	27 ± 4	5
Monomethyl succinate		
1	22 ± 17	30
5	54 ± 19	10*
10	125 ± 29	26*†
20	110 ± 20	5*†
Dimethyl succinate, 10	109 ± 22	8*†
Glyceraldehyde phosphate, 4, plus glucose, 5.5	203 ± 68	10*†

Values are means ± SD. *n* = number of observations.
*$P < .001$ vs. no addition.
†$P < .001$ vs. 5.5 mM glucose.
‡$P < .001$ vs. 1 mM glyceraldehyde.

xylulose 5-phosphate, erythrose 4-phosphate, and glycerol also failed to stimulate insulin release. Of these agents, only glyceraldehyde phosphate potentiated insulin release in the presence of a concentration of glucose (5.5 mM) that was at, or slightly above, the threshold of glucose for stimulating insulin release.

Although the intracellular penetration of dicarboxylic acids is poor, esters of dicarboxylic acids are taken up by some tissues (7). Monomethyl succinate and dimethyl succinate demonstrated potent insulin-releasing activity. These esters were equal in potency to leucine and were more stimulatory than 5.5 mM glucose. At a concentration of 10 mM, the following citric acid cycle intermediates, cycle precursors, or esters of carboxylic acids were not insulinotropic: citrate, isocitrate, 2-ketoglutarate, succinate, fumarate, malate, oxalacetate, glutamine, triethyl citrate, monoethyl succinate, dimethyl fumarate, monoethyl fumarate, and both the methyl and ethyl esters of pyruvate and lactate. Insulin release by glyceraldehyde phosphate is not a result of its breakdown in the media to an insulinotropic compound. Glyceraldehyde phosphate is degraded to methylglyoxal (8), which is not insulinotropic (data not shown). Similarly, stimulation of insulin release by methyl esters of succinate is not due to the methanol formed in the hydrolysis of the esters, because methanol does not stimulate insulin release (data not shown).

Preliminary studies of glyceraldehyde phosphate and of monomethyl succinate have not precisely discerned the key sites or process by which these compounds stimulate insulin release. These studies suggest that each secretagogue's activity is related to its metabolism in mitochondria, because inhibitors of mitochondrial metabolism, e.g., antimycin A and rotenone, inhibit insulin release by either secretagogue. However, a significant portion of glyceraldehyde phosphate's insulinotropic activity might be pharmacologic rather than primarily metabolic, because glyceraldehyde phosphate–induced insulin release is not as sensitive to metabolic inhibitors as that of glucose or monomethyl succinate. For example, 10 nM rotenone completely inhibits insulin release by either of the two latter compounds, but 100 nM to 1 μM rotenone inhibits glyceraldehyde phosphate–induced insulin release by no more than 50%. One micromole per liter antimycin A completely inhibits glucose- or monomethyl succinate–induced insulin release, but it inhibits insulin release by glyceraldehyde phosphate by 50% or less. Glyceraldehyde phosphate's insensitivity to these two inhibitors is a property shared by its congener glyceraldehyde (data not shown).

Glyceraldehyde phosphate, like glucose, is probably metabolized via the clearly defined pathway of aerobic glycolysis. Like the other glycolytic intermediates, glyceraldehyde phosphate is a highly charged compound. Thus, the reason glyceraldehyde phosphate alone should be capable of penetrating the cell membrane as well as stimulating insulin release is unknown. Glyceraldehyde phosphate's insulinotropism may be solely due to its far superior cellular penetrability over that of other intermediates. Although this possibility remains to be tested, it seems unlikely because the electrical charge of glyceraldehyde phosphate should be similar to that of other monophosphate intermediates. The failure of glycolytic intermediates above and below glyceraldehyde 3-phosphate to stimulate insulin release plus its relative insensitivity to certain inhibitors of mitochondrial metabolism may indicate that in addition to its possible metabolic effects, glyceraldehyde phosphate may have a key pharmacologic effect in stimulus-secretion coupling. Preliminary studies suggest the direct stimulation of inositol trisphosphate formation is one possible effect. Glyceraldehyde phosphate was the only glucose metabolite that consistently, albeit weakly, stimulated inositol trisphosphate formation in permeabilized islets [137 ± 10% ($n = 6$) for 0.5 mM glyceraldehyde phosphate vs. 100 ± 8% ($n = 18$) with no addition, means ± SE; $P < .01$; $K_{0.5} = 25$ μM]. In addition, several possible metabolites of glyceraldehyde phosphate, e.g., 2,3-diphosphoglycerate and fructose 1,6-bisphosphate, have been shown to strongly inhibit inositol trisphosphate breakdown (9) and potentiate inositol trisphosphate–induced calcium release from detergent-permeabilized islets (10).

Another possible explanation for glyceraldehyde phosphate's insulinotropism is that it may be situated at the last branch point in glucose metabolism that feeds all pathways that are necessary for stimulus-secretion coupling. Glyceraldehyde phosphate can be converted to pyruvate, which would be metabolized in the citric acid cycle. Glyceraldehyde phosphate could also increase the level of dihydroxyacetone phosphate (11), which, along with in-

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creased NADH formed in the glyceraldehyde phosphate dehydrogenase reaction, should activate the glycerol phosphate shuttle that is important for carbohydrate-induced insulin release, as indicated by the 50-fold–higher activity of the mitochondrial glycerol phosphate dehydrogenase in islets compared with other tissues (4). Intermediates distal to glyceraldehyde phosphate in the glycolytic pathway could not generate the substrates for this shuttle, because the NAD-to-NADH ratio in islets would not favor reversal of the reaction catalyzed by glyceraldehyde phosphate dehydrogenase, and reversal of this reaction would further increase the NAD-to-NADH ratio (12–14). This limitation would also prevent the conversion of these distal metabolites to possible effectors of inositol trisphosphate formation (glyceraldehyde phosphate itself) or degradation (fructose 1,6- and 2,6-bisphosphate and glucose 1,6-bisphosphate). The formation of only one inhibitor of inositol trisphosphate degradation, i.e., 2,3-diphosphoglycerate from metabolites distal to glyceraldehyde phosphate, might be possible. Glyceraldehyde phosphate cannot be formed from pyruvate, because islets lack phosphoenolpyruvate carboxykinase (15), which is necessary for forming phosphoenolpyruvate. This may explain why pyruvate, which is well metabolized in islets (16,17), and esters of pyruvate are not insulin secretagogues.

The insulinotropism of glyceraldehyde phosphate and of esters of succinate vaguely imply a dependence on site II of the mitochondrial electron-transport chain for insulin release. Succinate dehydrogenase transfers electrons to ubiquinone (site II). Glyceraldehyde phosphate that is converted to dihydroxyacetone phosphate and ultimately to glycerol phosphate in the cytosol should lead to stimulation of the mitochondrial glycerol phosphate dehydrogenase, which also transfers electrons to ubiquinone. A critical importance of site II could explain why esters of succinate, unlike esters of fumarate, are insulinotropic. However, because rotenone, an inhibitor of NADH dehydrogenase and of site I of the mitochondrial respiratory chain, inhibits insulin release by monomethyl succinate, insulin release by monomethyl succinate might also be related to increased mitochondrial NADH. The partial inhibition of glyceraldehyde phosphate–induced insulin release by rotenone might occur by way of glyceraldehyde phosphate's metabolism to pyruvate and the subsequent oxidation of pyruvate in the citric acid cycle. However, the possible source of NADH that results from succinate metabolism remains an enigma. The failure of malate and pyruvate, and pyruvate methyl ester and dimethyl fumarate, unlike methyl esters of succinate, to stimulate insulin release suggests that the source of intramitochondrial NADH might not be the dehydrogenases of the citric acid cycle.

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