

Elusive Proximal Signals of β -Cells for Insulin Secretion

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The β -cell is unique because its major agonists, i.e., insulin secretagogues, undergo metabolism instead of interacting with a receptor. This perspective presents the hypothesis that the first part of a metabolic signal of a secretagogue is specific to the secretagogue and the β -cell and can be envisioned as proximal. The second part, which occurs after transduction to more universal signaling mechanisms, is viewed as distal. Distal signaling and exocytosis in the β -cell operate the same as in other cells. Aerobic glycolysis is required for glucose-induced insulin release. Because glyceraldehyde, which enters metabolism at the triose phosphates in the glycolytic pathway, is a potent insulin secretagogue but pyruvate, which is metabolized in the mitochondrion, is not an insulin secretagogue, the proximal signal for glucose-induced insulin release originates with an interaction between the central part of the glycolytic pathway and mitochondrial metabolism. The proximal message in leucine-induced insulin release originates with leucine allosterically activating glutamate dehydrogenase, which activates endogenous glutamate metabolism, and by the metabolism of leucine itself. The methyl ester of succinate is a potent experimental insulin secretagogue. It is puzzling why the glucose signal requires the interplay of glycolysis and mitochondrial metabolism, whereas the signals from leucine and succinate originate entirely from within the mitochondrion. Leucine-induced insulin release is suppressed and glucose-induced insulin release is activated in islets cultured at a high concentration of glucose. Conversely, leucine-induced insulin release is activated and glucose-induced insulin release is suppressed in islets cultured at low glucose. We have correlated suppression of the insulinotropism of leucine and glucose with decreased expression

of the genes that encode the catalytic subunit of the first component of the branched-chain ketoacid dehydrogenase complex and the pyruvate dehydrogenase complex, respectively. This indicates that the proximal signal is specific to the secretagogue, whereas distal signals are more universal and are shared by many secretagogues. The proximal signaling mechanisms have yet to be elucidated, but many distal mechanisms are known. *Diabetes* 39:1461–66, 1990

β -CELL: UNIQUE FUEL SENSOR

The intracellular signal for insulin release by the β -cell is probably unique among endocrine, exocrine, nerve, muscle, and other excitatory tissues because it begins with metabolism of the major agonists. In all other tissues in mammals, the stimulus for excitation and/or contraction begins with the interaction of an agonist with a receptor. For example, nerve and muscle are highly active metabolically, but the signal for excitation in these tissues involves agonist-receptor interactions. In the β -cell, a metabolic signal must be converted to agonist-receptor interactions that instruct contractile processes to move insulin granules to the plasma membrane, where they are extruded into the circulation. This perspective addresses a unique feature of the β -cell, i.e., that it has discrete proximal metabolic signals for hormone release. I believe that the distal part of the signal for insulin release and exocytosis has a great deal in common with the same processes in many different types of cells.

Admittedly, the terms *proximal* and *distal* may create an artificial division between events that might occur concurrently rather than sequentially. Nevertheless, because something has to set in motion the events leading up to the exocytotic process, it is useful to think of the earliest events as proximal. The distal part of signal transduction, which is probably less specialized, is perhaps more pharmacological than metabolic. I envision the proximal signal as different from and occurring before the production of energy for the contractile processes of exocytosis. Distal or universal mes-

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sengers or processes include Ca^{2+} , inositol phosphates, G proteins, cAMP kinases, calmodulin kinases, protein kinases C, ATP-ADP and NADH-NAD ratios, and K^+ and Ca^{2+} channels. These factors are mentioned briefly in this perspectives, but factors that are believed to modulate insulin release, e.g., catecholamines, prostaglandin E_2 , somatostatin, glucagon, cholecystokinin, and galanin, are not discussed.

WHAT IS KNOWN ABOUT THE SIGNAL

The most potent physiological insulin secretagogue is glucose. Leucine is perhaps the second most potent. Other potent metabolizable initiators of insulin release that are useful experimentally are glyceraldehyde and the methyl ester of succinic acid (1–3). We are only certain about the first stages of metabolism of any of these secretagogues.

Glucose-induced insulin release. Aerobic glycolysis is important for glucose-induced insulin release, i.e., metabolism through the classic Emden-Meyerhof pathway and then through mitochondrial pathways, presumably the Krebs cycle. The hexose monophosphate shunt is not believed to be quantitatively important, but it may have some modulatory influence. Undoubtedly, glucokinase, which is present only in the liver and the β -cell (4–6) and has a K_m for glucose of 5–10 μM (which is in the range of the normal concentration of glucose in the blood), has a role as a glucose sensor. Hexokinase, which has a low K_m for glucose of $\sim 50 \mu\text{M}$, can also catalyze phosphorylation of glucose by ATP forming glucose-6-phosphate. Just how the β -cell is functionally or anatomically compartmentalized so that phosphorylation of glucose is catalyzed by glucokinase is not known, but it may involve channeling of glucose directly from the glucose transporter to glucokinase (7).

The first part of the glycolytic pathway from glucose transport and phosphorylation to the triose phosphates can probably be viewed as not essential in generating key messengers that initiate the signal for insulin release (1,8–10), because glyceraldehyde, which enters metabolism at the central part of the glycolysis pathway at the level of the triose phosphates, is a potent stimulator of insulin release, even more than glucose on the basis of triose equivalents.

Mitochondrial metabolism is important for glucose-induced insulin release because insulin release is prevented by inhibitors of oxidative phosphorylation. On the other hand, mitochondrial metabolism alone, which generates much more ATP than glycolysis and is responsible for oxidizing virtually all of the NADH produced by a cell, appears to be insufficient for glucose-induced insulin release, because pyruvate, the final metabolite of glycolysis and thus the first metabolite for the mitochondrion, does not stimulate insulin release, although it is metabolized well by islets. This mitigates the idea of energy production being the key proximal signal. Furthermore, ATP and ADP levels do not change much during a glucose stimulus (11–13).

Theoretically, pyruvate derived from glucose can enter mitochondrial metabolism by any of three routes: 1) by its carboxylation to malate in the cytosol catalyzed by malic enzyme, 2) by its carboxylation to oxalacetate in the mitochondrion catalyzed by pyruvate carboxylase, or 3) by its decarboxylation to acetyl-CoA catalyzed by pyruvate dehydrogenase in the mitochondrion. Islets contain each of

these enzymes. Recent evidence implicates pyruvate dehydrogenase as one of the enzymes involved in glucose metabolism. This enzyme is activated by glucose within 2 min of the application of glucose to islets (14).

Because pyruvate does not stimulate insulin release, glycolysis has important functions beyond substrate provision for the Krebs cycle. Pyruvate cannot be converted to phosphoenolpyruvate because islets lack phosphoenolpyruvate carboxykinase. Also, glyceraldehyde cannot signal insulin release by its conversion to fructose-6-phosphate or glucose-6-phosphate, because islets lack fructose biphosphatase, one of the enzymes necessary for the formation of hexose monophosphates from triose phosphates. Therefore, the central part of the glycolysis pathway from the triose phosphates to phosphoenolpyruvate has to be the segment that either interacts with mitochondrial metabolism in a special way and/or independently produces messengers to complement those produced by the mitochondrion. Figure 1 (*black box*) depicts this interaction as generating unknown proximal messengers.

Glycerol phosphate shuttle. One link between the triose phosphates and mitochondrial metabolism may be the glycerol phosphate shuttle. This suggestion is based on the fact that the activity of the mitochondrial glycerol phosphate dehydrogenase is 40- to 70-fold higher in the islets than in other tissues of the rat (8) and that, in humans, its activity is much higher in insulinomas and islets than in other tissues (15,16). In the glycerol phosphate shuttle, the triose phosphate dihydroxyacetone phosphate oxidizes NADH. This reaction, which is catalyzed by cytosolic glycerol phosphate dehydrogenase, forms glycerol phosphate that is in turn reoxidized to dihydroxyacetone phosphate by the mitochondrial glycerol phosphate dehydrogenase, an entirely different enzyme from the cytosolic enzyme with the same name. Most of the NADH is probably formed from glucose metabolism at the step in glycolysis catalyzed by glyceraldehyde phosphate dehydrogenase.

The mitochondrial dehydrogenase lies in the outer surface of the inner mitochondrial membrane, making it unnecessary for glycerol phosphate to enter the mitochondrion. The enzyme ensures the unidirectionality of the shuttle, because it transfers electrons directly to the electron transport chain at ubiquinone. Because pyridine nucleotides do not permeate the mitochondrial inner membrane, this provides a means of oxidizing cytosolic NADH. The β -cell has at least one other hydrogen shuttle to oxidize cytosolic NADH, and the capacity of this shuttle, the malate-aspartate shuttle, appears to be much higher than that of the glycerol phosphate shuttle, as judged from assayable enzyme activities (8,17). This implies that the glycerol phosphate shuttle must have a role beyond that of simply maintaining a normal NADH-NAD ratio in the cytosol.

One probably over simplistic explanation for the highly active glycerol phosphate shuttle in islets is that, in addition to facilitating glucose metabolism by oxidizing cytosolic NADH, it could enhance the rate of pyruvate metabolism by stimulating the uptake of Ca^{2+} into the mitochondrion. Metabolism of substrates by mitochondria is accompanied by Ca^{2+} uptake into mitochondria. In islet mitochondria, pyruvate does not stimulate Ca^{2+} uptake to nearly the extent that

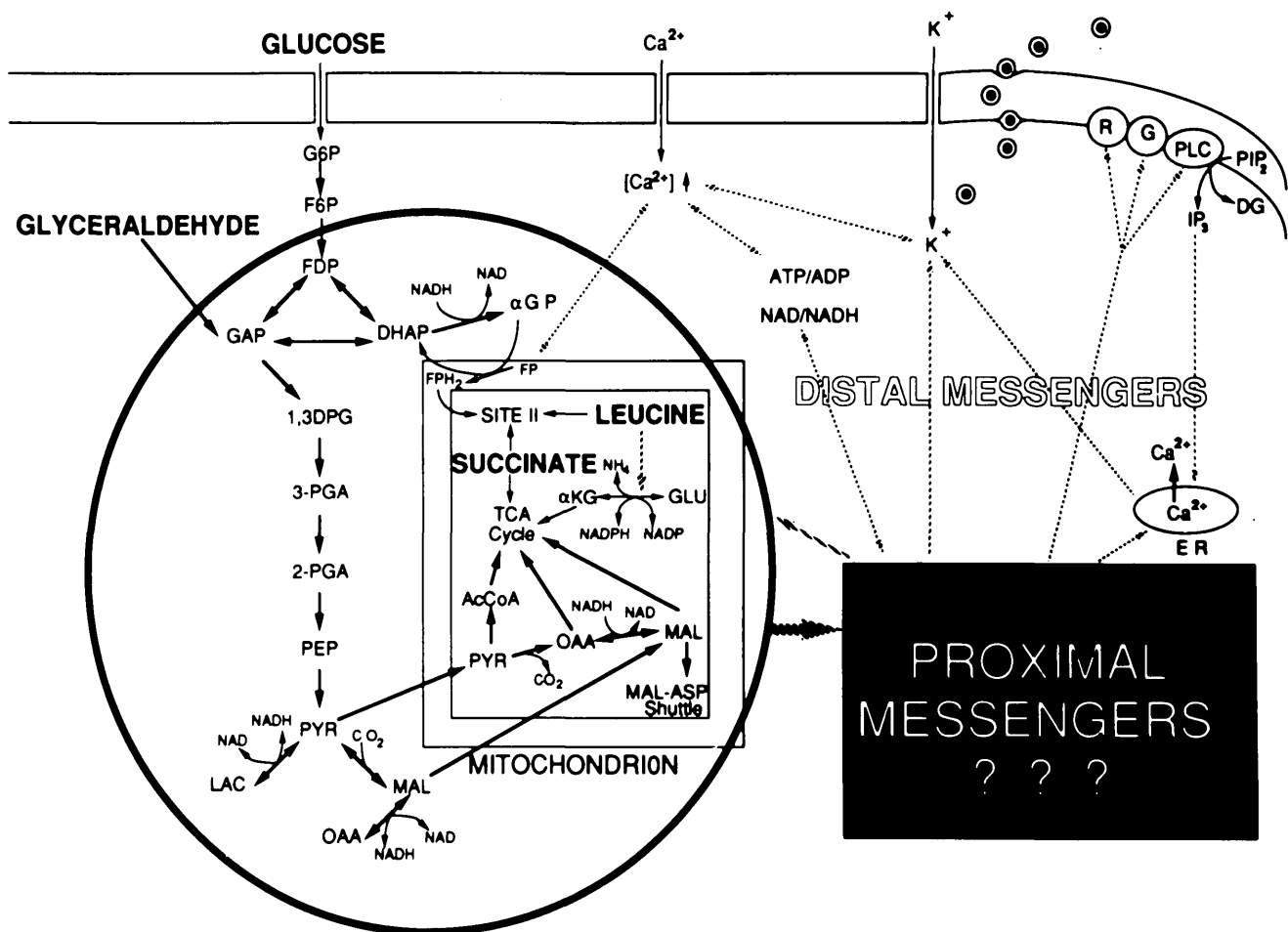


FIG. 1. Theoretical schema of metabolism-induced insulin release in β -cell. Metabolic pathways in large circle are depicted to generate specific proximal messengers that activate distal messengers shared by many secretagogues and many types of cells. Metabolic effects of 2 physiological insulin secretagogues, glucose and leucine, and 2 naturally occurring secretagogues not found in the blood, glyceraldehyde and succinate, are shown. Solid arrows, metabolic pathways or movement of ions; broken arrows, activation of process; R, receptor; G, G protein; PLC, phospholipase C; ER, endoplasmic reticulum.

glycerol phosphate or succinate do (18). An increase in mitochondrial Ca^{2+} activates enzymes in the matrix necessary for pyruvate metabolism, i.e., pyruvate dehydrogenase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase. This still does not explain what special messengers might be produced by glycolysis and mitochondrial metabolism.

Interestingly, another mitochondrial enzyme that is activated by Ca^{2+} is glycerol phosphate dehydrogenase (19). Presumably, because of its location, this enzyme is responsive to fluctuations in cytosolic Ca^{2+} . Thus, if glucose metabolism can increase cytosolic Ca^{2+} by increasing Ca^{2+} uptake from the extracellular space (perhaps by glycolytically generated ATP [20–25], glycolytic intermediates [22,26,27], other factors acting on plasma membrane ion channels, or glycolytic intermediates enhancing the release of Ca^{2+} from the endoplasmic reticulum [9,28]), then it can regulate the activity of the glycerol phosphate shuttle. The shuttle in turn might regulate glucose metabolism by the cytosolic NAD-NADH ratio and possibly by modulating the activities of mitochondrial enzymes.

Mitochondrially metabolized secretagogues. One of the most intriguing issues surrounding insulin release is why the interaction of extramitochondrial events with intramitochondrial events is required for glucose-induced insulin release but not for other insulin secretagogues. Several insulin secretagogues are metabolized entirely inside the mitochondrion. Leucine, which occurs physiologically, and succinate (as its methyl ester; 1,3), a classic substrate for mitochondrial studies, are each ~50% as insulintropic as glucose. Leucine probably stimulates insulin release via two intramitochondrial mechanisms. One mechanism is by allosterically activating glutamate dehydrogenase, a mitochondrial matrix enzyme (29). (Reference 2 cites evidence for this mechanism from original investigators' reports.) That is why glutamine, which is converted to glutamate by glutaminase and is not an insulin secretagogue by itself, can potentiate leucine-induced insulin release. This enhances the oxidative deamination of glutamate, which forms the ammonium ion NADPH and α -ketoglutarate, which can be metabolized in the Krebs cycle. What happens next and how it generates a signal for insulin release are not known.

We recently obtained evidence for a second mechanism for the insulinotropism of leucine. Leucine-induced insulin release, but not leucine plus glutamine-induced insulin release or glucose-induced insulin release, is suppressed completely by culturing islets in the presence of 20 mM glucose for 1 day, whereas glucose-induced insulin release is suppressed and leucine-induced insulin release is preserved in islets cultured at 1 mM glucose (30). High glucose suppresses by 90% the enzyme activity of and the mRNA encoding the catalytic subunit of the first enzyme of the branched-chain ketoacid dehydrogenase, a three-enzyme complex that catalyzes the first committed step in leucine metabolism. These studies suggest that leucine-induced insulin release is regulated by a negative glucose-responsive element in the gene for this enzyme (31). This kind of regulation could provide the small amount of insulin release needed to maintain fuel homeostasis when blood glucose and glutamine levels are below stimulatory levels and leucine is increased as in fasting.

Incidentally, our unpublished data indicate that the mRNA encoding the catalytic subunit of the first component of pyruvate dehydrogenase and the activity of this enzyme respond in a manner opposite to that of the branched-chain ketoacid dehydrogenase, i.e., they are increased at high glucose. This suggests that the insulinotropism of glucose is regulated similarly to that of leucine but by a positive glucose-response element located in the gene that expresses a catalytic subunit of pyruvate dehydrogenase. When blood glucose levels are normal, this gene should be activated.

WHAT IS NOT A PROXIMAL SIGNAL

A key issue in the study of insulin release is identifying what primary messengers are generated by secretagogue metabolism. It is unlikely that any single metabolite of a secretagogue is a potent messenger in signal transduction. Glucose metabolites have been the most studied. We found no striking insulinotropic effect of any intermediate of the glycolytic pathway between glucose-6-phosphate and pyruvate when they were applied to isolated islets in millimolar concentrations (10). Malate and fumarate by themselves or as their methyl esters had no effect. Glyceraldehyde 3-phosphate seemed to stimulate the first phase of insulin release and increase the formation of inositol trisphosphate (10). There is also evidence that 2,3-diphosphoglycerate and fructose-1,6-bisphosphate can inhibit degradation of inositol trisphosphate and sustain higher Ca^{2+} release from the endoplasmic reticulum (9,28). Glucose-6-phosphate has been reported to increase sequestering of Ca^{2+} in the endoplasmic reticulum, which would serve as an "off" signal (32), or in oscillations of Ca^{2+} . We believe that, although these effects of metabolic intermediates are real, they are primarily modulatory because they act on distal processes.

Fatty acids. The exact role of fatty acids in insulin release remains an enigma because fatty acids such as palmitate, the major fatty acid in the blood, do not stimulate insulin release from isolated pancreatic islets. Paradoxically, islet mitochondria contain a great deal of carnitine palmitoyltransferase, the enzyme that transports fatty acids into mitochondria for oxidation (33). The presence of enzymes necessary

for fatty acid oxidation has also been demonstrated in pancreatic islets (34). Several investigators have suggested that intraislet endogenous fatty acid metabolism modulates insulin release (34–38). How this might be accomplished requires a description too complex for this perspective.

WHERE SHOULD A SEARCH FOR THE SIGNAL BEGIN?

Any search for the glucose-signaled messengers should encompass the idea of glycolytically derived messengers or activity acting in conjunction with mitochondrially generated messengers. In the light of the fact that some insulin secretagogues are metabolized entirely intramitochondrially, glycolytically derived messengers may not be essential for signaling, but they might have a strong potentiating effect on primary mitochondrial messengers or processes. This would explain why glucose and glyceraldehyde are more potent insulin secretagogues than secretagogues that are metabolized purely by the mitochondrion. It may be that the specific messengers of the β -cell produced by glucose or glyceraldehyde metabolism are different from those generated by leucine and succinate. Several suggestions have been made that glycolytic factors, e.g., glycolysis-derived ATP (20–22) or glyceraldehyde phosphate and 2,3-diphosphoglycerate (25), have effects on membranes in smooth muscle cells, including on ion movements, whereas mitochondrial factors, e.g., the large amount of ATP generated by the mitochondrion, provide the energy needed for contraction (20–22). There are no a priori reasons why the glycolytic and mitochondrial messengers need to be as well known as ATP or glycolytic or citric acid cycle intermediates.

The reciprocal effects of culturing islets at low and high glucose on glucose- and leucine-induced insulin release indicate that at least the first parts of their signals are different. The time course of 1 day for the activation and suppression of insulin release by these secretagogues is consistent with altered rates of synthesis and degradation of enzymes required for metabolism of the secretagogues (30). Because the insulin release response to one secretagogue was "on" when the other was "off" and vice versa, all processes distal to the proximal signals must be constitutive and not regulated under the conditions we imposed on the islets. The suppressed leucine-induced insulin release is explained by the suppression by glucose of the gene expressing the first committed enzyme in the pathway of leucine metabolism (31). Identifying the site of suppression of glucose-induced insulin release might identify an important part of the proximal signal for glucose-induced insulin release. Until recently, we had been able to observe a difference in either mRNA levels or enzyme activities (or both) of various enzymes involved with glucose metabolism or exocytosis (Table 1). We think that we have identified this site as the catalytic subunit of the first component of the pyruvate dehydrogenase complex. The mRNA that encodes this enzyme and its enzyme activity are almost completely suppressed in islets cultured at low glucose. Thus, suppression and activation of the insulinotropism of glucose and leucine are regulated by genes that express the catalytic subunit of the first component of two very similar mitochondrial enzyme complexes.

Because secretagogues do not change ATP and ADP concentrations significantly over time, oscillations in these and

TABLE 1
List of enzymes not responsible for activation or suppression of glucose-induced insulin release in cultured islets

	mRNA	Enzyme activity
Glycolytic or cytosolic enzymes		
Glucokinase	✓	✓
Glucose-6-phosphate dehydrogenase	✓	
6-Phosphogluconate dehydrogenase	✓	
Glyceraldehyde phosphate dehydrogenase	✓	✓
Phosphoglycerate mutase	✓	
Pyruvate kinase		✓
Glycerol phosphate dehydrogenase (cytosolic)	✓	
Malic enzyme	✓	✓
Quinone reductase	✓	✓
Phosphoenolpyruvate carboxykinase	ND	ND
Glutathione S-transferase (4 isoenzymes)	✓	
Mitochondrial enzymes		
Glycerol phosphate dehydrogenase		✓
Pyruvate carboxylase	✓	
Glutamate dehydrogenase	✓	✓
Succinate dehydrogenase		✓
Fumarase	✓	
Enzymes involved in exocytosis		
Ca ²⁺ -calmodulin-dependent protein kinase II	✓	
Protein disulfide isomerase	✓	
Myosin light-chain kinase	✓	

✓, <50% difference in mRNA and/or enzyme activity was detected; ND, not detectable in islets. Glucose-induced release is activated in islets cultured for 24 h at 20 mM glucose and suppressed in islets cultured at 1 mM glucose. There is evidence that activation and suppression are due to enzyme (de)induction (30). Because net synthesis of enzymes listed above does not change between high and low glucose, (de)induction of these enzymes is not responsible for activation or suppression. Enzyme activity and/or mRNA of enzymes were measured in islets cultured for 24 h at either 1 or 20 mM glucose.

other factors may be important. Oscillations in β -cell electrical activity, glycolysis (39), adenine and pyridine nucleotides, and Ca²⁺ and K⁺ membrane currents might all be interconnected. If the ATP-ADP ratio oscillates, then the NADPH-NADP ratio must also oscillate. Since the first documentation of secretagogue-induced alterations in NADPH in islets (40), many studies have been directed at the redox issue. All of these studies hint at the importance of the redox potential, but how it is linked to the signal is still not known. It is possible, however, that oscillations are important for no more than the relaxation and recocking of the contractile processes of exocytosis.

How finely tuned changes in ATP/ADP, NADPH/NADP, or acetyl-CoA/CoA might influence insulin release is one of the most complex and unsettled questions. The membrane depolarization resulting from an increased ATP-ADP ratio inhibiting ATP-sensitive K⁺ channels might increase Ca²⁺ uptake (22–24,39). For this to occur, areas of low ATP localized at sites near the plasma membrane are necessary because these channels are inhibited at 0.2 mM ATP, and the overall intracellular ATP concentration is usually 4–5 mM. On the other hand, it has been proposed that a transient fall in the ATP-ADP ratio due to initial glucose phosphorylation may permit internal Ca²⁺ to rise by depressing the activity of membrane Ca²⁺-ATPases (41). Besides not knowing in what

direction ATP-ADP and NADPH-NADP ratios are going, it is unknown whether these events are a consequence or a cause of the signal for insulin release. Because some major secretagogues are metabolized intramitochondrially, the oscillating pacemaker might be tightly connected to mitochondrial metabolism. Each of the above-mentioned oscillating factors are changed by more than one secretagogue. Therefore, they may not be specific or proximal. Proximal factors are probably unknown.

In scientific inquiry, a grueling search to solve a mystery often never ends. As long as proximal factors are undiscovered, the search is alive and of heuristic value and can be expressed in the words of the Canadian poet and part-time gold prospector Robert Service (42)

There's gold, and it's haunting and haunting;
It's luring me on as of old;
Yet it isn't the gold that I'm wanting
So much as just finding the gold.

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