The Role of Intracellular Ca^{2+} in the Regulation of Gluconeogenesis

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A hypothesis for the hormonal regulation of gluconeogenesis, in which increases in cytosolic free-Ca^{2+} levels ($[Ca^{2+}]_i$) play a major role, is presented. This hypothesis is based on the observation that gluconeogenic hormones evoke a common pattern of Ca^{2+} redistribution, resulting in increases in $[Ca^{2+}]_i$. Current concepts of hormonally evoked Ca^{2+} fluxes are presented and discussed. It is suggested that the increase in $[Ca^{2+}]_i$ is functionally linked to stimulation of gluconeogenesis. The stimulation of gluconeogenesis is accomplished in two ways: (1) by increasing the activities of the Krebs cycle and the electron-transfer chain, thereby supplying adenosine triphosphate (ATP) and reducing equivalents to the process; and (2) by stimulating the activities of key gluconeogenic enzymes, such as pyruvate carboxylase. The hypothesis presents a conceptual framework that ties together two interrelated manifestations of hormone action: signal transduction and metabolism.
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THIS REVIEW CONCERNS the role that changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) play in the regulation of gluconeogenesis. Gluconeogenesis is the process by which the organism replenishes blood glucose when it is low, by producing it from noncarbohydrate precursors such as lactate, amino acids, and glycerol. In fasting, for instance, gluconeogenesis accounts for up to 96% of total glucose production.[1] It also contributes to elevating blood glucose in both insulin-dependent and non–insulin-dependent diabetics.[2-4] During exercise, higher levels of glucagon and reduced insulin secretion cause an increase in glycogenolysis and gluconeogenesis.[5]

The major steps in the pathway of gluconeogenesis, which occurs mainly in the liver, are well known. However, details regarding individual enzyme activities and methods for measurement of gluconeogenesis are still being investigated.[6-8] This complex process involves a partial reversal of the glycolytic pathway. In three steps, which are thermodynamically irreversible, namely at the conversion of (1) pyruvate to phosphoenolpyruvate, (2) fructose 1,6-bisphosphate to fructose-6-phosphate, and (3) glucose-6-phosphate to glucose, specific enzymes for gluconeogenesis perform the reactions. The pathway of gluconeogenesis spans several subcellular compartments. Thus, the conversion of pyruvate to phosphoenolpyruvate occurs—according to the species—partially in the mitochondria or in the cytosol, while the endoplasmic reticulum is involved in changing of glucose-6-phosphate to glucose.

Recent studies indicate additional levels of complexity; namely that the enzymes of the cytosol and the mitochondrial matrix exist within the cell in the form of multienzyme complexes. Studies by Berry et al[9] have shown that the enzymes of glycolysis in the liver might be segregated from those of gluconeogenesis. Besides associating with other enzymes, glycolytic enzymes also associate with cytoskeletal components such as microtubules.[10] Interactions among enzymes and between enzymes and cytoskeletal elements are likely to profoundly influence the characteristics of these proteins. These interactions also influence their kinetic properties. Thus, the pathway of gluconeogenesis includes three subcellular compartments, and the enzymes participating form, in part, multienzyme complexes including some that interact with cytoskeletal elements. Thus, at the intracellular level, we have a highly structured distribution of gluconeogenic enzymes. In addition, many studies indicate that functional separation of parenchymal cells exists at the level of the acinus, the structural and functional unit of the hepatic parenchyma.[11,12] Major enzymes for gluconeogenesis are preferentially expressed in cells at the periportal region of the lobule, whereas glycolytic enzymes are more abundant in pericentral areas. These characteristics of periportal and pericentral (or perivenous) cells are maintained in primary cell cultures[13,14] and are manifested in the human liver.[15] Recently, a description of a Percoll density-gradient centrifugation method was published that allows simultaneous preparation of periportal and perivenous cells.[16] A view of all these structural factors is important to understanding the regulation of the pathway, and to seeing its regulation by hormones.

The hormones that can stimulate gluconeogenesis include glucagon, epinephrine, and norepinephrine. These hormones are secreted in response to hypoglycemia. Glucocorticoids are secreted also in response to hypoglycemia and have both direct and "permissive" effects.[17] Thyroid hormone, vasopressin, and some growth factors also have stimulatory effects. Insulin is the physiological counterregulatory hormone. Stimulation of gluconeogenesis starts with the onset of certain changes that increase the flux toward glucose production using enzymes already present in the cell. Therefore, the acute phase of stimulation does not require de novo protein synthesis. An induction of key gluconeogenic enzymes follows.[18,19] This article reviews only events in the acute regulation of gluconeogenesis—it does not address the induction of enzymes.

While chemical steps in the pathway of gluconeogenesis are well established, there is no consensus as to how hormonal stimulation of the pathway is accomplished. The major differences in viewpoints relate to where the regulation occurs in the gluconeogenic pathway: namely the relative importance of the steps at the three reactions representing nonequilibrium steps in the glycolytic pathway, listed above. Early studies showed that the rate-

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limiting and flux-generating step is the first step in the pathway, namely pyruvate carboxylation.[20-24] In other studies, the importance of futile cycling at the pyruvate kinase level, with its inhibition by gluconeogenic hormones, was suggested as a regulated reaction.[25,26] Reexamining the role of pyruvate kinase inhibition reinforced earlier conclusions, ie, it is the activation of pyruvate carboxylation rather than the inhibition of pyruvate kinase that is important for the stimulation of glucose production from amino acids and lactate.[27] Pyruvate cycling is low in fasted livers.[6]

The concept that hormonal regulation of gluconeogenesis is accomplished by control mechanisms affecting futile substrate cycling, for instance, by inhibition of pyruvate kinase, or at the level of phosphofructokinase, represent a currently widely held view reflected in many review articles and textbooks. To quote from one of the reviews, "The fact that hepatocytes contain rate-controlling enzymes specific for gluconeogenesis and glycolysis, phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase (Fru-1,6-P_2ase), glucose-6-phosphatase (Glu-6-Pase), pyruvate kinase (PK), 6-phosphofructo-1-kinase (6-PF-1-K), and glucokinase (GK) makes a sensitive control system possible. Cycling of the substrates and products of these opposing reactions can be governed in rate and direction of net flux by changes in allosteric effectors, by changes in the concentration of the enzymes involved in the cycles, and/or by covalent modification of these enzymes. According to this view, three substrate cycles, each of which is driven by enzymes acting in opposite directions, determine whether the hepatocytes produce or utilize glucose."[26]

A cornerstone of this approach is fructose 2,6-bisphosphate, a metabolite generated in the liver. This substance is a potent stimulator of phosphofructokinase.[28] The level of this metabolite decreases in fasting and in diabetes to one tenth of the level present in normal rats. These changes correlated with the decreased rates of glycolysis and increased rates of gluconeogenesis that are also associated with fasting and diabetes. Points that need further clarification in connection with the function of substrate cycles include reports that the rates of glycolysis in the liver are negligible.[6,29] Substrate cycling might account for only 10% to 15% of the glucose formed by gluconeogenesis.[30,31] In some experimental conditions, such as sucrose-feeding, carbon flow through fructose 1,6-bisphosphatase remains high despite high levels of fructose 2,6-bisphosphate, which in vitro inhibit the enzyme.[32] Also, indications that glycolytic and gluconeogenic enzymes are separated structurally inside the cell, and that in the hepatic lobule some cells might be glycolytic while others are gluconeogenic, need to be reconciled with the model of futile substrate cycling as the primary factor in regulation of gluconeogenesis. Nevertheless, the concept that levels of fructose 2,6-bisphosphate and regulation of futile cycling are the primary determinants of gluconeogenesis is a current widely held view and has been described in detail.[26,33]

It is therefore the purpose of this article to review another aspect of gluconeogenesis: namely the role that Ca^{2+} plays in its regulation. This review is timely, in light of the progress made during the last 10 years in the elucidation of the signal transduction process and in the understanding of the role that Ca^{2+} plays in the regulation of gluconeogenesis. First, signal transduction by Ca^{2+} will be discussed. This will be followed by a description of how changes in Ca^{2+} distribution affect gluconeogenesis.

SIGNAL TRANSDUCTION

The onset of gluconeogenesis is signaled by hormones secreted in response to real or perceived (diabetes) hypoglycemia. The receptors sensing hypoglycemia are in the central nervous system and the pancreas. The physiologically important hormones that are secreted and stimulate gluconeogenesis are the catecholamines, glucagon, and glucocorticoids. Other hormones also stimulate gluconeogenesis; for instance, thyroid hormone and vasopressin. Vasopressin, in fact, is used as an experimental tool to study gluconeogenesis. These hormones will be mentioned in conjunction with the discussion of regulatory processes. Since the focal point in this article is the role of Ca^{2+} in the gluconeogenic process, the mechanism by which hormones affect Ca^{2+} distribution and increase cytosolic free-Ca^{2+} levels $[Ca^{2+}{}_i]$ will be presented according to the second messengers generated by the hormones, cyclic adenosine monophosphate (cAMP) and IP_3.

Every hormone that stimulates gluconeogenesis also increases $[Ca^{2+}]_i$.[17,34-36] One exception to this might be the stimulation of gluconeogenesis by glucocorticoids,[37] which will be discussed separately. A general description of the ways that the liver manages Ca^{2+} distribution has been previously reported.[34,36,38]

Glucagon, Catecholamines and cAMP

Glucagon interaction with the liver starts with the binding glucagon to a receptor on the cell surface. This receptor is a well-characterized protein consisting of a 45-kd peptide backbone and four *N*-linked glycans. It transects the plasma membrane.[39,40] After glucagon-binding to the receptor, the activation of adenylate cyclase—an event coupled by G-proteins—follows.[41,42]

The binding of catecholamines, acting through β-adrenergic receptors, also results in the activation of adenylate cyclase by a similar mechanism involving heterotrimeric G-proteins. The β-adrenergic receptor is a hydrophobic molecule, possessing seven stretches of hydrophobic amino acids arranged in seven transmembrane-spanning β-helices.[43] In addition to β-adrenergic receptors acting by generating cAMP, the liver also possesses α-adrenergic receptors localized both at the plasma membrane and in the intracellular membrane.[44] The major coupling system of the α_1-adrenergic receptor pathway seems to be the activation of phospholipase C via α-pertussis toxin–insensitive G-protein. However, in some instances, α_1-adrenergic receptor activation can also increase intracellular cAMP levels. This effect is especially evident in aged rats.[45,46] Male and female rats differ in their responses to catecholamines.[47] A recent review article that focuses on α-adrenergic actions discusses specific problems in need of resolution.[48] It is generally accepted that α-adrenergic effects are mediated by the activation of phospholipase C rather than by cAMP.

This will therefore be discussed together with the effects of other hormones that activate phospholipase C.

The first demonstration of glucagon-, epinephrine-, norepinephrine-, and cAMP-evoked Ca^{2+} redistribution was made in perfused rat livers.[49] These studies on Ca^{2+} as a potential modulator of key enzymes in the gluconeogenic pathway were initiated because of the demonstration that the onset of the gluconeogenic response to a stimuli is immediate and does not require de novo protein synthesis.[50] In these studies, preloaded Ca^{2+} was released from the liver upon hormonal stimulation. The release occurred equally in the presence or absence of extracellular Ca^{2+}, indicating that the release occurred from an intracellular Ca^{2+}-storage site and was not dependent on Ca^{2+} influx. The release almost immediately followed addition of the hormones, and was also observed in later studies, with a physiological concentration of glucagon.[51] Studies using electron-probe microanalysis of rapidly frozen liver samples indicated the endoplasmic reticulum as the source of Ca^{2+} release.[52] In earlier studies, a biphasic effect on K^+ fluxes was also noted, consisting of K^+ uptake followed by K^+ release. The release of K^+ is associated with hyperpolarization of the liver cell membrane.[17,53]

In addition to stimulating Ca^{2+} release, glucagon and cAMP also stimulate Ca^{2+} uptake into liver cells.[54] The data from Keppens et al on Ca^{2+} influx were confirmed in many different laboratories.[55-61] Thus, glucagon and cAMP were shown to make Ca^{2+} available to the cytoplasm from both intracellular and extracellular pools. Based on experiments in which increases in $[Ca^{2+}]_i$ following glucagon or cAMP were manipulated by addition of Ca^{2+} at different time intervals to the suspension medium, Bygrave et al[59] suggested that the influx of Ca^{2+} occurs earlier and precedes the mobilization of Ca^{2+} from intracellular stores. Activity of the plasma membrane Ca^{2+} pump does influence the amount of Ca^{2+} retained in the cell. A proteolytic fragment of glucagon was shown to alter the activity of plasma membrane Ca^{2+}-adenosine triphosphatase (ATPase), but the physiological importance of this finding for Ca^{2+} movement in the liver has yet to be confirmed.[62]

The advent of intracellular Ca^{2+} probes, allowing measurement of $[Ca^{2+}]_i$, heralded a new era for studies on the role of Ca^{2+} in cellular processes.[63,64] Until then, $[Ca^{2+}]_i$ could be measured only in very large cells by using Ca^{2+}-sensitive microelectrodes or by microinjection of probes. Measurement of $[Ca^{2+}]_i$ following glucagon administration demonstrated an increase in cytosolic free Ca^{2+} levels, as expected from data on the effects of glucagon on Ca^{2+} release and influx.[56,57,65-67] The glucagon receptor has been recently cloned and is able to bind glucagon and increase cAMP and cytosolic free Ca^{2+} levels.[40] These data confirm previous observations that indicated that activating the glucagon receptor leads to changes in Ca^{2+} fluxes, resulting in increased cytosolic free Ca^{2+} levels. However, the question as to whether the effects of glucagon are due to and secondary to increases in cAMP levels or are due to some additional effect of receptor activation was not resolved by these experiments.

In the majority of studies in which the effects of glucagon and cAMP were compared, the effects were similar: namely cAMP addition mimicked the effects of glucagon.[49,65,66] Glucagon-stimulated increases in $[Ca^{2+}]_i$ were mimicked in experiments where (Sp)-cAMPS, a synthetic analog of cAMP, binds to the regulatory subunit of the cAMP-dependent protein kinase, was used.[68] These results suggest that the effects of glucagon on $[Ca^{2+}]_i$ are mediated by the increase in cAMP. Relevant to these findings is the recent demonstration of the presence of mRNA for the retinal cyclic-nucleotide-gated Ca^{2+}-channel protein in liver.[69] In this study, preliminary Western blot analysis with monoclonal antibodies raised against the bovine rod channel α-subunit identified a polypeptide, indicating that the channel protein is likely to be present in the liver in a sufficient amount to be of physiological importance. However, isolated reports maintain that adding cAMP to liver cells does not increase $[Ca^{2+}]_i$.[70] It was also suggested that the liver contains two different types of receptors for glucagon. One, when occupied, results in increased cAMP levels, and another is coupled to the second-messenger, inositol 1,4,5-trisphosphate (IP_3).[71,72] In contrast to these findings, according to Poggioli et al,[58] glucagon had no effect on IP_3 formation. Pittner and Fain[73,74] also measured IP_3 levels following glucagon administration and did not find an increase. However, the effects of glucagon on Ca^{2+} mobilization are not always identical to that of cAMP.[75] Whether this indicates that glucagon might have additional effects to the activation of adenylate cyclase is not clear. Numerous reports indicate that glucagon augments Ca^{2+} responses obtained with hormones that act through another messenger, IP_3. This facet of glucagon action will be discussed following the discussion of IP_3 effects on $[Ca^{2+}]_i$.

While epinephrine was shown to stimulate both Ca^{2+} efflux and Ca^{2+} uptake into the liver (see above), the question of the relative potency of β-adrenergic agents and the question as to what extent the effects of epinephrine are due to α- or β-adrenergic stimulation are unsettled in the literature.[76] The synthetic catecholamine, isoproterenol, is considered the most potent β-adrenergic agonist in terms of its ability to stimulate production of cAMP. However, isoproterenol is far less effective than glucagon in elevating cAMP levels.[77,78] Reports on the effects of isoproterenol on $[Ca^{2+}]_i$ are conflicting. Isoproterenol by itself was reported to cause a small efflux of Ca^{2+} from preloaded livers, but it was said to evoke a massive efflux of Ca^{2+} when given after angiotensin II, a hormone shown to increase IP_3 levels in the liver.[79] In these experiments, as well as in experiments where the effects of isoproterenol on increases in $[Ca^{2+}]_i$ were evaluated in the same laboratory, the concentration of isoproterenol was 50 nmol/L. At this low concentration, isoproterenol produced inconsistent effects on $[Ca^{2+}]_i$.[80] However, the usually effective concentration of catecholamines is in the micromolar range. In fact, when the effect of isoproterenol on $[Ca^{2+}]_i$ was measured at 500 nmol/L, both isoproterenol and cAMP elevated $[Ca^{2+}]_i$, as indicated by measurements of Ca^{2+}-dependent K^+ conductance.[81] Indeed, isoproterenol (0.2 μg/mL) was shown to evoke a hyperpolarization of the liver cell membrane, similarly to cAMP, which was not inhibited by α-adrenergic blockers.[82]

Thus, the negative results obtained by Burgess et al[79,80] may well have been due to the low concentrations of the drug used in those studies.

In summary, glucagon and catecholamines were shown to profoundly alter Ca^{2+} distribution in liver cells by stimulating both the influx of Ca^{2+} and Ca^{2+} release from intracellular stores, thereby increasing $[Ca^{2+}]_i$. In the intact liver, propagation of the Ca^{2+} signal might involve gap junctions, as suggested by the finding of Combettes et al,[83] who reported that mechanical disruption can interfere with the Ca^{2+} signal. Although it is debated as to what extent some of the catecholamine effects are due to interaction with β- or α-receptors, both β- and α-adrenergic agents were shown to increase $[Ca^{2+}]_i$. In the majority of the studies, cAMP mimicked hormonal effects on $[Ca^{2+}]_i$. This supports the notion that the effects of these hormones are mediated by cAMP. The recent demonstration of the presence of mRNA for the cyclic-nucleotide–gated channel protein in the liver also favors the view that the effects of glucagon are mediated by cAMP, because it indicates that a cAMP-gated channel might be present in the liver.[69] However, the possibility that binding of glucagon to its receptor results in interaction with more than one G-protein cannot be excluded.[71,84]

α-Adrenergic Hormones and Vasopressin

Since the pioneering studies from the laboratories of Glinsmann and Fain,[77,85,86] it has become evident that catecholamine effects, exerted through α-adrenergic receptors, can be dissociated from increases in cAMP levels and are likely to be mediated by Ca^{2+}.[54,87] At about the same time, the effects of vasopressin and angiotensin II on hepatic carbohydrate metabolism were also studied. These hormones usually are present at levels that would not affect hepatic metabolism; however, in severe shock or trauma, the levels increase sufficiently to impact the liver. The vasopressin receptor in the liver is a glycoprotein coupled through binding to a specific G-protein to phosphoinositide metabolism.[87-90] It was also shown that the effects are not mediated by cAMP.[91]

The effects of these hormones on Ca^{2+} fluxes are similar to the effects of glucagon and β-adrenergic agents. They evoke a large-scale biphasic redistribution of ions.[92] Thus, like glucagon, they evoke an influx of Ca^{2+}.[60,93-95] It is interesting that gadolinium, an inhibitor of stretch-activated channels, also inhibits the hepatic divalent-cation inflow system.[95]

In addition to the entry of Ca^{2+}, vasopressin and α-adrenergic hormones also cause the release of Ca^{2+} from intracellular stores.[17,34,36,38] As with glucagon, it is assumed that the intracellular pool from which Ca^{2+} is released is the endoplasmic reticulum.[52] It was suggested by experiments in which glucagon and vasopressin were sequentially administered to perfused livers that the pools from which these two hormones release Ca^{2+} are identical.[51,96] The question remains: What is the nature of the Ca^{2+} release channel? This question will be discussed in conjunction with IP_3 action. Although both α-adrenergic agonists and vasopressin increase the cytosolic Ca^{2+} level, due to increases in IP_3 level, their effects on Ca^{2+} fluxes differ somewhat.[97] Subsequent to the Ca^{2+} influx and the release of Ca^{2+} from intracellular stores, $[Ca^{2+}]_i$ increases. The increase in $[Ca^{2+}]_i$ has an oscillatory nature.[98-102] Various models were proposed to explain these oscillatory responses. Thus, it could be attributed to the influx of Ca^{2+} or to its release, and these oscillatory responses also were attributed to the possible existence of either one or two Ca^{2+} pools or Ca^{2+}-induced Ca^{2+} release.[103,104] It was also suggested that calmodulin participates in the development of oscillatory responses.[101] Oscillation in Ca^{2+} content of the perfusate was observed in perfused livers following hormonal stimulation.[105] Whether these oscillations in Ca^{2+} content of the perfusate and oscillations observed in $[Ca^{2+}]_i$ are related and correlated is not clear. Oscillatory changes in $[Ca^{2+}]_i$ seem to be associated with oscillatory changes in NAD(P)H formation. This indicates that $[Ca^{2+}]_i$ might pace and direct metabolic functions.[106]

Following the increase in $[Ca^{2+}]_i$, according to Duddy et al,[107] the rate of Ca^{2+} efflux increases, which serves to terminate the Ca^{2+} signal. Also, to be discussed later, increases in $[Ca^{2+}]_i$ lead to the entry and uptake of Ca^{2+} into the mitochondria.[76] This phase of the Ca^{2+} signaling process is a key event in the metabolic responses evoked by hormonal stimulation.

It is generally agreed that the effects of these hormones on Ca^{2+} fluxes and the increase in $[Ca^{2+}]_i$ are the result of increases in the levels of IP_3, generated in response to hormonal stimulation.[108,109] Evidence that IP_3 increases cytosolic free Ca^{2+} levels was found in permeabilized cells exposed to IP_3. In these cells, administration of IP_3 was invariably followed by increases in $[Ca^{2+}]_i$.[110,111] In other experiments, it was shown that monoclonal antibodies, raised against membrane phosphoinositides and introduced into guinea pig hepatocytes, blocked generation of IP_3. In these cells, α-adrenergic agents failed to increase $[Ca^{2+}]_i$.[112] What is not agreed upon is the exact mechanism by which the generated IP_3 releases Ca^{2+} from intracellular pools. The controversial point is localization of the receptor for IP_3. This is the question: Is the receptor located on the endoplasmic reticulum and does it act there as a Ca^{2+} release channel, or is the IP_3 receptor located at the plasma membrane and function there as a Ca^{2+} influx channel?[113,114] If the receptor is on the endoplasmic reticulum and acts there as a Ca^{2+} channel,[108] then the well-demonstrated entry of Ca^{2+} has to occur through a different, yet-unidentified channel, and it is secondary to Ca^{2+} release. To explain Ca^{2+} release and Ca^{2+} entry, Putney and Bird[115] proposed the theory of "capacitative entry" as a link between these two processes. Accordingly, emptying of the intracellular store generates a signal that would cause opening of the plasma membrane channel allowing Ca^{2+} entry. The connection between store-emptying and Ca^{2+} entry found experimental support with the use of thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase. [116] According to a recent study by Duszynski et al,[117] an inward Ca^{2+} current is responsible for vasopressin-stimulated Ca^{2+} entry. Thapsigargin was shown to trigger the opening of this channel. Pertussis toxin treatment of

hepatocytes impaired their ability to respond with Ca^{2+} influx to store-emptying, indicating a possible role for G-proteins in the process.[118] The possibility of a calcium influx factor, which couples store-emptying to Ca^{2+} influx, was recently proposed,[119,120] but it is too early to know its relevance to the liver. It was also suggested that adenine-guanine nucleotide levels have an effect on capacitative Ca^{2+} influx: decreasing the level of either nucleotide was reported to decrease Ca^{2+} influx.[121]

On the other hand, experiments in which the binding of IP_3 to high-affinity binding sites was measured demonstrated that IP_3 binding sites copurified with the plasma membrane fraction rather than the endoplasmic reticulum–derived microsomal fraction[122]; additional references are listed in other studies.[36,113,114] The IP_3 receptor seemed to be attached to cytoskeletal elements, although the exact nature of the protein to which the IP_3 receptor is anchored was not determined.[113,123] Based on binding data obtained in our own and in many other laboratories, we proposed that the IP_3 receptor might act as a Ca^{2+} influx channel, because the IP_3 receptor was shown to have Ca^{2+} channel properties.[124] Support for this proposal is provided by the finding that microinjection of IP_3 into hepatocytes stimulated the influx of Ca^{2+}, an effect directly attributed to IP_3.[93,125] Moreover, an IP_3 receptor protein was recently purified from the liver plasma membrane fraction, which, when incorporated into lipid bilayers, exhibited Ca^{2+} conductance.[126] These data support the notion that the plasma membrane IP_3 receptor is a Ca^{2+} influx channel.[113,114]

In the liver, an additional IP_3 binding site is present at the nuclear fraction, which is a different isoform than the plasma membrane receptor.[127] The receptor at the nucleus might be involved in regulation of nuclear Ca^{2+} levels and thereby might influence Ca^{2+}-dependent nuclear processes. In a recent study, antibodies against the C-terminal residues of the brain type I receptor were used for immunolocalization of the hepatic IP_3 receptor.[128] In our laboratory, antibodies against the brain type I receptor interacted with the nuclear fraction, but not with the plasma membrane or smooth endoplasmic reticulum fractions. Therefore, further studies are needed with antibodies that can distinguish between various isoforms of the receptor to localize the different types of IP_3 receptors in the liver. Currently, it is not known whether the nuclear receptor is involved in regulation of $[Ca^{2+}]_i$, and therefore it will not be discussed here.

If, indeed, as the binding data and studies by Mayrleitner et al[126] indicate, the IP_3 receptor is at the plasma membrane, then a different receptor may trigger release of Ca^{2+} from intracellular storage pools. We proposed, based on binding studies, that this channel is a ryanodine-binding protein in the endoplasmic reticulum–derived microsomal fraction of the liver. Ryanodine is a plant alkaloid used in the past as an insecticide; studies on its mechanism of action revealed that it affects Ca^{2+} flux and stimulates O_2 uptake in insects. Subsequently, ryanodine was successfully used to explore the excitation-contraction process in muscle cells. The receptor for ryanodine is a Ca^{2+} channel. Various isoforms of the receptor protein were found to be expressed in a number of organs.[129-131] We reported the presence of high-affinity ryanodine binding sites in the microsomal fraction. Moreover, addition of ryanodine to liver cells mimics the effects of gluconeogenic hormones, by stimulating O_2 uptake and gluconeogenesis. Ryanodine also activates glycogen phosphorylase.[122,132-134] The hepatic ryanodine receptor is a different protein from the so-far-identified ryanodine receptor isoforms, as indicated by the fact that it does not interact with antibodies generated against skeletal or cardiac muscle receptors.[132] According to a recent study, none of three so-far-identified isoforms of the ryanodine receptor are present in the liver, or at least not in easily detectable quantities.[131] However, some structural similarities between hepatic and skeletal muscle ryanodine receptors are indicated by a recent study in our laboratory. The immunosuppressant, FK506, which binds to a low–molecular-weight receptor protein, was shown to interact with skeletal muscle ryanodine receptor. In the liver, FK506 was shown to decrease ryanodine binding.[135] Further studies are needed for molecular characterization of the Ca^{2+}-release channel.

Synergistic Effects Between Glucagon, Vasopressin, and α-Adrenergic Agents on Ca^{2+} Fluxes

In studies where α-adrenergic agents or vasopressin were added to glucagon and the combined effects of the hormones on various parameters of Ca^{2+} distribution were measured, synergistic effects of these two classes of hormones were noted. Thus, Ca^{2+} influx when the two types of hormones were added together was greater than when each type of hormone was added alone.[35,136] Simultaneous administration of α-adrenergic agents with glucagon to Fura-2–loaded hepatocytes resulted in synergistic stimulation of Ca^{2+} oscillations. The effects of cAMP mimicked the effects of glucagon on Ca^{2+} oscillations.[137]

Various mechanisms were proposed to explain the synergistic effects of glucagon or cAMP with hormones that increase IP_3 levels. There are conflicting reports as to whether glucagon influences IP_3 production by hormones that act by activating phospholipase C. According to Poggioli et al,[54] glucagon addition did not increase the production of IP_3 either by itself or in combination with either vasopressin or epinephrine. On the other hand, Pittner and Fain[138] reported that glucagon enhanced vasopressin-stimulated formation of IP_3. Because the two types of hormones release Ca^{2+} from the same intracellular pool,[51,96] Ca^{2+} influx is the likely determinant of the additive effect. This notion is supported by the observation that in the absence of extracellular Ca^{2+} in quin-2–loaded hepatocytes, glucagon administration no longer enhanced the maximal increases in $[Ca^{2+}]_i$ to vasopressin.[139]

Two recent studies suggested that cAMP-activated protein kinase either activates the IP_3 receptor by phosphorylating it[140] or phosphorylates the receptor, thereby increasing its open, Ca^{2+}-conducting state.[141] However, recent demonstrations of the presence of both an IP_3-gated Ca^{2+} channel in the plasma membrane[126] and mRNA for a cyclic-nucleotide–gated channel protein in the liver[69] might offer a mechanistic basis for the additive effects. Hormones that

act by increasing IP_3 levels activate Ca^{2+} influx through the IP_3-gated channel. Glucagon might activate Ca^{2+} influx through the cyclic-nucleotide–gated channel. Such two second-messenger–activated pathways were reported to operate in lobster olfactory neurons.[142] Thus, when the two types of hormones are added simultaneously, two channels open. This could account for the observed additive effects.

Hormonal Effects on Volume Regulation

It has been noted in many studies that adding hormones to the liver, such as glucagon, vasopressin, and cAMP, decreases cell volume, whereas adding not only insulin but also phenylephrine increases hepatocyte cell volume. It was therefore suggested that changes in cell volume might act as a cellular signal, linking metabolism to hormonal and environmental alterations.[143] In terms of the signal by which changes in a cellular hydration state affect cellular function, a number of potential mechanisms were considered, including changes in $[Ca^{2+}]_i$.[144-146] Because $[Ca^{2+}]_i$ is a suggested mediator of the effects of volume changes on metabolism, this topic is briefly covered here.

Cells invariably respond to changes in the osmolarity of their surrounding medium with profound adaptive changes. The major reason for the adaptive changes was suggested to be preservation of the concentration of cytosolic macromolecules.[147] Indeed, in the liver, upon fasting, despite an almost 50% decrease of water content, the concentration of water and that of cellular proteins hardly change.[148] The question is, then, are the changes in cell volume observed in hepatocytes an essential link in the chain of events that lead to the increased rate of gluconeogenesis? This question was examined by Gaussin et al.[149] In their experiments, the times required for changes in intracellular water space, cAMP accumulation, stimulation of phosphorylase a activity, inactivation of pyruvate kinase, and decrease in fructose 2-6-bisphosphate content were compared. Results of these experiments indicated that volume changes follow rather than precede metabolic changes. A lack of coupling between cell volume and gluconeogenesis is indicated also by data showing that agents that have differing effects on gluconeogenesis affect cell volume in the same way. Thus, insulin, which decreases gluconeogenesis, causes cell swelling, but so does phenylephrine, an α-adrenergic agent that stimulates gluconeogenesis or alanine, a physiological precursor for gluconeogenesis.[143] Although it is likely that changes in cell volume have far-reaching consequences for liver cell functions, the accumulated data do not warrant the conclusion that hormonally induced changes in cell volume are a prerequisite for increased gluconeogenesis.

Unresolved Questions With Respect to Hormonal Effects on Ca^{2+} Fluxes

Despite extensive studies, major questions remain in connection with the mechanism by which hormones elevate $[Ca^{2+}]_i$. These questions relate to how Ca^{2+} is released from intracellular stores, how Ca^{2+} influx can be activated, how we connect these two processes, and how cytosolic Ca^{2+} oscillations happen. Thus, in connection with glucagon and cAMP, while it is agreed that both glucagon and cAMP increase the uptake of Ca^{2+}, the channel responsible for the influx has not been characterized. The recent description of the presence of mRNA for a cyclic-nucleotide–gated channel need to be followed up with studies on the channel protein. There is disagreement as to whether glucagon actions are due solely to the increases in cAMP, or whether some glucagon effects can be attributed to changes like those of IP_3 levels. Glucagon also releases Ca^{2+} from the endoplasmic reticulum. This effect is not due to inhibition of uptake. We propose that the release is through a different, yet-unidentified channel, which might have some similarities to ryanodine-binding channels. The molecular nature of the channel has yet to be identified, and the mechanism by which the channel is activated has yet to be elucidated. Cyclic-ADP-ribose was suggested as an endogenous regulator of nonmuscle-type ryanodine channels,[150] but whether this type of regulatory mechanism applies to the liver remains to be seen. The following additional questions need to be answered: If the first event is Ca^{2+} influx, by what mechanism is Ca^{2+} release initiated? And what is the role of cytoskeleton or caveolae,[151] if any, in glucagon-induced increases in $[Ca^{2+}]_i$?

Similar questions need to be addressed with respect to the mechanism by which hormones that elevate IP_3 levels act. Some of these questions were discussed in detail in previous articles.[36,38,114] Thus, localization of the hepatic nonnuclear IP_3 receptor has to be firmly established. If the receptor is indeed at the plasma membrane, gating Ca^{2+} influx,[126] the molecular identity of the channel at the intracellular Ca^{2+} storage pool has to be proven, and the mechanism by which IP_3 releases Ca^{2+} from the endoplasmic reticulum has to be elucidated.

ROLE OF Ca^{2+} IN THE REGULATION OF GLUCONEOGENESIS

Three lines of evidence indicate a role for Ca^{2+} in the regulation of gluconeogenesis: (1) every hormone that increases gluconeogenesis evokes a change in Ca^{2+} fluxes; (2) inhibition of Ca^{2+} fluxes blocks the stimulation of gluconeogenesis; and (3) increasing $[Ca^{2+}]_i$ is sufficient to increase gluconeogenesis.

Each of the above points will be discussed briefly, followed by the mechanism by which an increase in cytosolic Ca^{2+} stimulates gluconeogenesis.

Every Hormone That Increases Gluconeogenesis Evokes a Change in Ca^{2+} Fluxes

Glucagon, epinephrine, norepinephrine, vasopressin, and angiotensin II share a common pattern on Ca^{2+} distribution. This pattern is characterized by stimulation of Ca^{2+} influx, release of stored Ca^{2+} from an intracellular pool, and the associated elevation of $[Ca^{2+}]_i$, as described in the previous section.

Other hormones that stimulate gluconeogenesis also affect Ca^{2+} fluxes. It was demonstrated over 20 years ago that thyroid hormone increases Ca^{2+} influx into the liver.[152] In more recent studies, those initial observations were confirmed. Triiodothyronine-induced Ca^{2+} uptake and stimulation of gluconeogenesis had similar time courses,

indicating that the changes in Ca^{2+} influx and the increase in glucose production are related.[153] Diiodothyronine also causes a rapid stimulation of oxygen uptake, as do all other gluconeogenic hormones.[154] Several other hormones were reported to increase $[Ca^{2+}]_i$ and rates of gluconeogenesis. These include pancreastatin,[155] epidermal growth factor,[156] histamine, and prostaglandins.[157-160] Two studies were performed by Yamaguchi on the effects of calcitonin on liver metabolism.[161,162] The liver is not considered a target of calcitonin action, a hormone whose physiological target organs are the bone and the kidney. Yamaguchi used calcitonin as an experimental tool. He reported that injection of calcitonin to rats increased glucose output by the liver that was parallel to the increased Ca^{2+} uptake by the cells. Purinergic receptor activation by ATP, ADP, and GTP in the liver also leads to a biphasic change in $[Ca^{2+}]_i$: an initial increase due to Ca^{2+} influx, followed by a plateau due to release from the intracellular pool. These changes in Ca^{2+} fluxes were associated with a twofold increase in the rate of gluconeogenesis.[163]

These are examples, then, of a wide variety of agents acting through different receptors, which share the characteristics of increasing $[Ca^{2+}]_i$ and stimulating glucose production by the liver.

Perusal of the data available on hormonal effects of Ca^{2+} fluxes show that although all these hormones evoke a similar pattern of Ca^{2+} fluxes, quantitative differences were reported between the pattern of Ca^{2+} distribution. In our hands, for instance, glucagon and norepinephrine[49] or glucagon and vasopressin[51] caused Ca^{2+} effluxes from the liver that were quantitatively similar. On the other hand, other laboratories reported greater increases in $[Ca^{2+}]_i$ after vasopressin administration than after glucagon administration.[56] Thus, there is no quantitative correlation between the magnitude of the hormonal effect on $[Ca^{2+}]_i$ and the stimulation of gluconeogenesis. This is especially evident in the case of ryanodine, a plant alkaloid that we used as an experimental tool, and therefore will be discussed in conjunction with it.

Inhibition of Ca^{2+} Fluxes Blocks the Stimulation of Gluconeogenesis

Having described the hormone-induced ion fluxes in the liver (reviewed in Friedmann[17,164]), our laboratory examined the relevance of these ion fluxes to the stimulatory effect of the hormones on gluconeogenesis. Of these early studies, only one is described here.[165] In this study, two experimental conditions—which were previously shown to inhibit the effects of glucagon and cAMP on Ca^{2+} fluxes and gluconeogenesis—were reexamined to test whether their inhibitory effects are due to inhibition of the activation of protein kinases by cAMP. These conditions were as follows: use of Na^+-free perfusate or administration of the local anesthetic, tetracaine. Neither experimental condition resulted in the inhibition of protein kinase A activation by cAMP. Thus, a possible conclusion that one can derive from these data is that when Ca^{2+} fluxes are blocked, activation of protein kinase A is not sufficient to produce stimulation of gluconeogenesis, and both effects are equally important. In contrast, Connelly et al[68] suggested that protein kinase A activation is the primary manifestation of glucagon stimulation of adenylate cyclase, and that Ca^{2+} is increased only at high levels of glucagon. This view is shared by other investigators.[56] Nevertheless, based on our own findings, namely that we could not dissociate between the manifestation of these two effects, and the fact that agents that affect Ca^{2+} fluxes but do not activate protein kinase A, such as cGMP,[56,165] increase gluconeogenesis, we suggested that Ca^{2+} fluxes are an integral and essential link in the chain of events leading to the metabolic effects evoked by glucagon. In subsequent studies, we demonstrated that tetracaine, incorporated into liposomes and administered intravenously to rats, inhibited the hyperglycemia evoked usually by epinephrine injection. Moreover, tetracaine vesicle injection was capable of decreasing the blood glucose of diabetic rats.[166] Tetracaine also blocked the metabolic responses to vasopressin.[109] Although tetracaine has more than one effect, in this case the effects of tetracaine might be due to its interaction with the endoplasmic reticulum ryanodine-binding Ca^{2+}-release channel, thereby interfering with the Ca^{2+} response, because tetracaine inhibits ryanodine binding.[122,167] Other inhibitors of ryanodine-binding in the liver, dantrolene and diltiazem,[132] block gluconeogenic responses to ATP[163] and to glucagon.[168] These experiments and others like these provide further evidence as to the essential role that Ca^{2+} plays in the elicitation of metabolic responses.

Increasing $[Ca^{2+}]_i$ Is Sufficient to Increase Gluconeogenesis

We have postulated[17,49,165] that, to a large extent, changes in Ca^{2+} distribution are responsible for increases in glucose production observed in the liver after administration of gluconeogenic hormones. Although all the gluconeogenic hormones elicit this effect, to test this hypothesis, we used ryanodine, an agent that acts by interacting with a Ca^{2+}-channel protein.[129,130] Presently, the interaction of ryanodine with specific Ca^{2+}-channel proteins is considered the mechanistic basis of its effects. Therefore, the effects of ryanodine on $[Ca^{2+}]_i$, O_2 uptake, and gluconeogenesis were measured. Even though the hepatic receptor is different from the known Ry Rs, it does interact with FK506BP, similarly to the skeletal muscle RyR.[135,169] Ryanodine increased $[Ca^{2+}]_i$ in the liver, as indicated indirectly by activation of glycogen phosphorylase a and by direct measurement of $[Ca^{2+}]_i$ in fura-2–loaded hepatocytes.[134,135] Ryanodine administration to perfused livers evoked an increase in O_2 consumption. Indeed, the effect of ryanodine on O_2 uptake was its first recognized effect in muscle. This effect was not caused by direct effects of ryanodine on mitochondria, because addition of ryanodine directly to liver mitochondria had no effect on O_2 uptake. Thus, the increased O_2 uptake is secondary to effects of ryanodine on $[Ca^{2+}]_i$. Ryanodine also increased gluconeogenesis by the liver.[133] The effects of ryanodine on $[Ca^{2+}]_i$ are slower in onset and smaller in magnitude than the hormonal effects. This again raises the question as to the lack of quantitative correlation between increases in the level of $[Ca^{2+}]_i$ and the stimulation of gluconeogenesis. Despite the modest effect

of ryanodine on $[Ca^{2+}]_i$, the stimulation of O_2 uptake following ryanodine administration to the perfused liver is comparable in magnitude to the hormonal stimulation of O_2 uptake. Because of this observation, it seems that it is not the absolute level of $[Ca^{2+}]_i$, but rather the degree of stimulation of mitochondrial O_2 uptake and metabolism, that correlates with the stimulation of gluconeogenesis. Further studies are needed to establish this point.

In summary, we have listed a wide variety of hormones and pharmacological agents that stimulate gluconeogenesis in the liver. The common denominator in the action of these agents is their ability to affect cellular Ca^{2+} fluxes, increase O_2 uptake, and stimulate gluconeogenesis. Inhibition of Ca^{2+} fluxes was shown to inhibit gluconeogenesis. This suggests that increases in $[Ca^{2+}]_i$ in the liver are a causative link in the chain of events leading from receptor occupation to glucose production.

Mechanism by Which Elevated $[Ca^{2+}]_i$ Increases Gluconeogenesis

Four consequences of elevated $[Ca^{2+}]_i$ that contribute to the increases in gluconeogenesis will be discussed: (1) stimulation of respiration, (2) stimulation of pyruvate carboxylase activity, (3) stimulation of phosphoenolpyruvate carboxykinase (PEPCK) activity, and (4) activation of Ca^{2+}-dependent protein kinases.

1. Gluconeogenic hormones were shown to stimulate respiration. The stimulatory effect of glucagon on mitochondrial respiration was described first by Yamazaki et al.[170] The stimulatory effect of glucagon on a key gluconeogenic enzyme localized in the mitochondria, pyruvate carboxylase, was previously reported.[171] These and subsequent early studies were covered in a previous review article.[17] A detailed discussion of the hormonal effects on mitochondrial metabolism was written by Hoek.[172] Here, therefore, only selected points are discussed.

Although there are conflicting data in the literature about the effects of hormonal treatment on mitochondrial Ca^{2+} content, for instance, in one study glucagon pretreatment increased mitochondrial Ca^{2+} while pretreatment with vasopressin did not,[173] it is the accepted view today that the increase in O_2 uptake is linked to the increase in $[Ca^{2+}]_i$. The increase in intramitochondrial Ca^{2+} levels results in the activation of several intramitochondrial dehydrogenases,[174,175] although an initial decrease in pyruvate dehydrogenase activity was also reported.[176] The activity of the electron-transport chain also increases. The exact mechanism by which this happens is still debated. It might be due to volume-mediated changes, as suggested by Quinlan and Halestrap.[177] The increase in respiration is Ca^{2+}-dependent, but it does not require the influx of extracellular Ca^{2+} into the cell as long as enough Ca^{2+} is stored inside the cell to activate the process.[178] In addition to activation of the respiratory chain and the Krebs cycle, Ca^{2+} also influences intramitochondrial adenine nucleotide pool size by influencing the activity of ATP-Mg/Pi cotransporter.[179] Adenine nucleotide pool distribution is influenced also by the activity of adenine nucleotide translocase. It was shown that hormones that act by increasing cAMP levels influence the activity of adenine nucleotide translocase.[180]

The increases in Krebs cycle activity and O_2 uptake stimulate gluconeogenesis by generating ATP and by providing reducing equivalents to the cytosol. This latter process was shown to be both Ca^{2+}- and energy-dependent.[181-184] The increase in O_2 uptake indirectly stimulates pyruvate uptake into the mitochondria. Stimulation of O_2 uptake also invariably leads to inhibition of glycolytic enzyme activities (the Pasteur effect). Activation of the tricarboxylic acid cycle also was shown to be essential for fatty acid stimulation of gluconeogenesis.[185]

Related to these effects is a Ca^{2+}-dependent regulatory mechanism suggested by Sterniczuk et al.[186] It was postulated in that study that after hormonal stimulation, the Ca^{2+} taken up by mitochondria decreases the K_m of 2-oxoglutarate, which leads to its increased utilization and stimulation of the malate-aspartate cycle. Stimulation of 2-oxoglutarate dehydrogenase activity could be especially important for stimulation of gluconeogenesis from glutamine and proline.

Further evidence to support the critical role that increases in O_2 uptake play in the regulation of gluconeogenesis was obtained using inhibitors of the respiratory chain.[187-189] The inhibitory effects of these agents are caused by decreasing ATP/ADP ratios and the redox state of pyridine nucleotides. Moreover, oxygen supply per se was suggested to play a decisive role in both acute and long-term regulation of gluconeogenesis.[190,191] As pointed out already, liver cells located in the periportal zone of the lobule are rich in gluconeogenic enzymes, and cells in the perivenous zone are rich in glycolytic enzymes. Evolvement of this pattern might be largely due to differences in O_2 supply. Accordingly, hypoxia was shown to decrease gluconeogenesis.[192] Thus, one can conclude that both types of experiments—those in which increased respiration correlated with increased gluconeogenesis and those in which inhibition of respiration decreased gluconeogenesis—support the notion that increased respiration has a decisive role in its stimulation.

2. Stimulation of pyruvate carboxylase activity—the first and flux-generating enzyme in the gluconeogenic pathway—by a low concentration of Ca^{2+} (0.25 nmol/mg mitochondrial protein) was demonstrated based on experiments in which hepatic Ca^{2+} content was increased by treatment with calcitonin.[161,162] In these experiments, a small but significant increase in mitochondrial Ca^{2+} content and a large stimulation of gluconeogenesis were observed. The role of Ca^{2+} in the stimulation of pyruvate carboxylase was also studied by Walajtys-Rhode et al.[193] In this detailed study, pyruvate carboxylase and pyruvate dehydrogenase activities were measured in a variety of experimental conditions using various inhibitors to characterize parameters affecting these reactions. The results demonstrated a correlation between mitochondrial Ca^{2+} content and pyruvate carboxylation. However, the investigators concluded that this correlation is not due to a direct effect of Ca^{2+} on pyruvate carboxylase, which might be secondary to in-

creases in ATP/ADP and NADH/NAD^+ ratios affecting flux through the pyruvate dehydrogenase complex also. In earlier studies, Halestrap and Armston[194] attributed the activation of pyruvate carboxylase to increases in the ATP/ADP ratio. Thus, probably indirectly, increases in intramitochondrial Ca^{2+} in the physiological range result in increased pyruvate carboxylation.

3. Stimulation of phosphoenolpyruvate synthesis by Ca^{2+} activation of PEPCK, the second enzyme in the gluconeogenic pathway, was reported by Deaciuc et al.[195] This study used guinea pig liver mitochondria, in which, as in human liver, this enzyme is present both inside the mitochondria and in the cytosol. Synthesis of phosphoenolpyruvate in this study was attributed to stimulation of a dehydrogenase by Ca^{2+}. In another study, Titheradge et al[196] suggested that a hormone-induced decrease in 2-oxoglutarate content also stimulates phosphoenolpyruvate generation. Thus, Ca^{2+}, acting indirectly, affects the activity of the enzyme, PEPCK, in such a way that synthesis of phosphoenolpyruvate is increased.

4. Activation of Ca^{2+}-dependent protein kinases by gluconeogenic hormones was suggested in early studies from the laboratory of Garrison,[197] which reported on the phosphorylation of many hepatic proteins following administration of gluconeogenic hormones. Mieskes et al[198] reported that Ca^{2+}-dependent phosphorylations are due to Ca^{2+}/calmodulin-dependent protein kinases, which phosphorylated several enzymes especially relevant to "futile cycling" points in the pathway, for instance, L-type pyruvate kinase.[199] Thus, phosphorylation of crucial enzymes by Ca^{2+}/calmodulin-dependent protein kinases was shown to inhibit "futile cycling" and facilitate carbon flux toward the synthesis of glucose. These hormonal effects on protein phosphorylation have been reviewed extensively.[26,33]

Effects of Glucocorticoids

Glucocorticoids exert two types of effects on the liver: so-called "permissive" effects and direct effects.[17] The permissive effects are likely to be extrahepatic and are due to the essential role that glucocorticoids play in the release of amino acids—precursors for glucose—from muscle. In the liver, glucagon, β-adrenergic agents, and cAMP were demonstrated to stimulate the proteolytic process[200] and to inhibit protein synthesis. Adrenalectomy interferes with these effects, indicating that glucocorticoids do play a role in the regulation of hepatic protein metabolism also.[201,202] Thus, glucocorticoids play a decisive role in providing precursors for glucose production, both from muscle and from the liver itself. In addition, glucocorticoids also have a lipolytic effect in the liver.[203] The increase in lipid breakdown provides free fatty acids for oxidation, glycerol for gluconeogenesis, and reducing equivalents. This process is also influenced by glucocorticoids. Thus, glucocorticoids affect a wide range of hepatic and extrahepatic processes, each related to and influencing gluconeogenesis.

Glucocorticoids were also shown to be able to acutely stimulate gluconeogenesis. In a recent study, treatment of starved rats for 90 minutes with dexamethasone, a synthetic glucocorticoid, increased glucose output in subsequently isolated hepatocytes.[37] The increase in glucose output was attributed to increased flux through pyruvate carboxylase, and it was suggested that the acute effects of glucocorticoids to increase gluconeogenesis reside at the level of pyruvate carboxylase and PEPCK activities. Previous studies indicated that there might also be a different pattern of protein phosphorylation in adrenalectomized rats.[165,204]

Relevant to this review is the question of whether some of the acute effects of glucocorticoids can be due to some changes in Ca^{2+} distribution. In earlier studies, livers from adrenalectomized rats were shown to respond to gluconeogenic hormones by a normal release of Ca^{2+}. Dexamethasone by itself had no immediate effect on Ca^{2+} release.[49] Livers from adrenalectomized rats were able to respond to gluconeogenic hormones by the changes in membrane potential observed in normal rats.[205] However, at the subcellular level or when increases in $[Ca^{2+}]_i$ were measured, adrenalectomized rats were reported to show impairments. Thus, in adrenalectomized rats, after glucagon injection, the usually observed increases in Ca^{2+} retention time in mitochondria decreased by 50%. Dexamethasone injection before glucagon treatment restored the response. This restoration of response was inhibited by inhibitors of protein synthesis.[206] Dexamethasone was also shown to induce microsomal Ca^{2+}-ATPase, potentially influencing the amount of Ca^{2+} stored intracellularly.[207] Reflecting these changes in intracellular Ca^{2+} sequestration, adrenalectomized rats were reported to respond to adrenergic stimuli less effectively than control animals in terms of increases in $[Ca^{2+}]_i$, an effect attributed, at least partially, to a decrease in IP_3 accumulation.[208]

The findings that in adrenalectomized rats hepatic mitochondria have a defective response to gluconeogenic hormones in terms of Ca^{2+} retention might relate to the observations that glucocorticoids affect the activities of pyruvate carboxylase and PEPCK, two Ca^{2+}-dependent enzymes. This might provide a functional explanation for the acute effects of glucocorticoids.

CONCLUSION

A hypothesis is presented in which a role of Ca^{2+} in regulating gluconeogenesis is suggested. This hypothesis was constructed based on these observations: (1) every hormone that stimulates gluconeogenesis alters Ca^{2+} distribution, (2) interfering with the Ca^{2+} distribution blocks the stimulation of gluconeogenesis, and (3) increasing cytosolic free Ca^{2+} levels by itself can lead to stimulation of gluconeogenesis. The suggested role for Ca^{2+} seems to be especially relevant to gluconeogenesis from precursors that enter the pathway at the level of the mitochondria, and thus from amino acids and lactate. For substrates entering the pathway after the mitochondrial steps, such as glycerol, inhibition of futile cycling might be more important.

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