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SIGNAL TRANSDUCTION IN PANCREATIC β-CELLS: REGULATION OF INSULIN SECRETION BY INFORMATION FLOW IN THE PHOSPHOLIPASE C/PROTEIN KINASE C PATHWAY

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1. ABSTRACT

The physiologic regulation of glucose-induced insulin secretion is dependent upon the activation of information flow in the phospholipase C (PLC)/protein kinase C (PKC) signal transduction system. In both rat and human pancreatic β-cells, glucose has several time-dependent effects on secretory responsiveness including the regulation of biphasic insulin secretion, time-dependent potentiation and time-dependent suppression. PLC/PKC activation has been implicated in all three response patterns. Islets of Langerhans contain the three major PLC isozyme classes (β1, γ1 and δ1) and the available evidence suggests that one class is activated by fuel secretagogues and another by neurohumoral agonists. The expression and activation of PLC is labile. When rat islet are cultured for short periods, the content and activation of PLC in response to glucose decreases and this biochemical defect in signal transduction is paralleled by significant reductions in glucose-induced insulin secretion. Similar defects are observed when human islets are cultured as well. Mouse islet responses to glucose stimulation differ in several major and dramatic ways from rat and human islet responses. When stimulated by 15mM glucose, mouse islets fail to develop a rising second phase secretory response, they fail to exhibit either time-dependent potentiation or time-dependent suppression to the hexose. Biphasic insulin secretion can be evoked and time-dependent potentiation can be induced in mouse islets by carbachol, an agonist that activates an isozyme of PLC distinct from that activated by glucose. These divergent response patterns are attributable to the underexpression in mouse islets, when compared to rat islets, of a fuel-sensitive PLC isozyme. When taken in their entirety, the experimental evidence suggests that the activation of PLC is an essential component in the physiologic regulation of insulin secretion and that disordered activation of the enzyme culminates in disordered insulin release.

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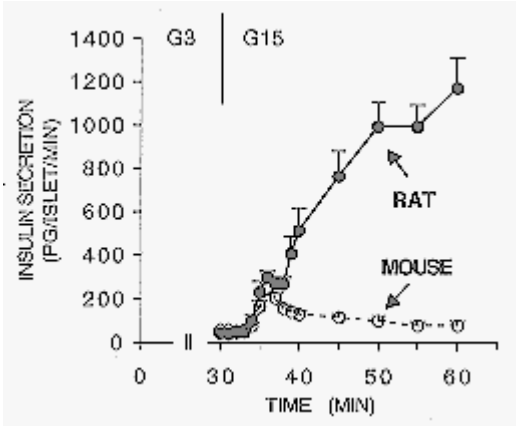

Figure 1. Biphasic Glucose-induced Insulin Secretion from Perifused Rat Islets but not Perifused Mouse Islets. Groups of rat (closed circles) or mouse (open circles) islets were collagenase isolated and perifused with 3mM glucose (G3) for 30 minutes prior to stimulation with 15mM glucose (G15). Note the characteristic biphasic insulin secretory response characterized by a rising second phase evoked from rat islets but the flat sustained response of mouse islets.

2. INTRODUCTION

The maintenance of glucose homeostasis depends upon a well orchestrated series of hormonal, metabolic, ionic and second messenger signaling events. At the level of the pancreatic β-cell, these processes ensure that the release of insulin will be commensurate to satisfy peripheral tissue requirements for the hormone and, as a consequence of its pleiotropic effects on a variety of target tissues, maintain fuel homeostasis. Failure at any level in this cascade of β-cell signals puts the organism at risk for glucose intolerance, a pathognomonic clinical complication we commonly associate with Type II noninsulin dependent diabetes (NIDDM). It is not the purpose of the present review to revisit the multiple signaling events which occur in peripheral tissues such as liver and muscle which respond to insulin or to the postulated gut (incretin) factors, such as glucagon-like peptide-1 or gastric inhibitory peptide, which participate in the physiologic regulation of insulin secretion. Rather the scope of this review is intentionally narrow with a primary focus on how glucose and other secretagogues activate the pancreatic β-cell and the role of increased information flow in the phospholipase C (PLC)/ protein kinase C (PKC) signaling system in the regulation of insulin secretion. This is a most dynamic area of investigation and it is one that is not without significant controversy as well. Reasons for the discrepancies which exist in the literature will be addressed so that the reader may form their own conclusions as to the relevance of the reported results to the physiologic regulation of insulin secretion. At the outset, however, it is important to review the multiple and diverse effects that glucose has on the pancreatic β-cell. The comments that follow will deal primarily with the time-dependent actions that glucose exerts on insulin secretion. It should be borne in mind, however, that additional effects on insulin biosynthesis and β-cell enzyme induction have also been described.

3. MULTIPLE EFFECTS OF GLUCOSE ON THE PANCREATIC β-CELL

3.1 Acute Regulation of insulin release.

The release of insulin from the pancreatic β-cell is dependent upon the interaction of a variety of signaling molecules with β-cell signal transduction systems

(1-
3)

. It is commonly accepted that glucose is the primary physiologic regulator of β-cell sensitivity and that it exerts multiple effects on β-cell response patterns. At postprandial concentrations, it independently

increases insulin secretion. Sustained, short-term (1 hour or less) glucose stimulation of rat islets results in a biphasic insulin secretory response (Figure 1) characterized by a transient first phase of release and a slowly rising and sustained second phase response that is, depending on the preparation and the glucose level, 30-60 fold greater than prestimulatory release rates

(9, 10)

(4-8)

and quantitatively

. A qualitatively

similar biphasic insulin secretory response to sustained glucose stimulation has been noted from human β-cells studied with the hyperglycemic clamp methodologies, observations which lend credence to the concept that the biochemical signaling events which regulate insulin secretion from rat β-cells are similar, if not identical, to the events which occur in human β-cells. Thus it is not unreasonable to extrapolate findings made with rat islets to human islets. As will become evident later, similar extrapolations with mouse β-cells must be cautiously made since pronounced species differences, which have been ignored in many instances, exist in the patterns of glucose-induced insulin secretion from mouse islets.

3.2 Fail-safe regulation of insulin secretion and time-dependent potentiation

In addition to stimulating a biphasic insulin secretory response, glucose also performs the role of a fail-safe regulator of secretion. For example, at glucose levels greater than 6mM acetylcholine (11, 12) or cholecystokinin (CCK) (13)

(14)

(1)

are potent stimulants for secretion but at hypoglycemic levels they fail to activate insulin exocytosis from the β-cell. This, so-called permissive effect of glucose, is thought to be a result of glucose metabolism and the provision of adequate amounts of ATP to support a secretory response

. The fact that other nutrient secretagogues that are well metabolized supply the same permissive signals supports this concept

(15)

(4, 7, 16, 17)

. Glucose stimulation of the β-cell also sensitizes it to subsequent restimulation with a variety of agonists

. This response is induced by prior short-term stimulation with glucose and manifests itself in the heightened release of insulin during subsequent restimulation. Termed time-dependent potentiation (TDP), this phenomenon can be induced by agonists as structurally diverse as acetylcholine

(19)

(18)

, α-ketoisocaproate

, cholecystokinin

(20)

, monomethylsuccinate

, glyceraldehyde
(15)

(21)
, methyl pyruvate

(22)

(23, 24)

and, perhaps most importantly, by the PKC activator tetradecanoyl phorbol acetate (TPA)

. TDP may play a particularly important role in cephalic phase insulin secretion where vagally-derived acetylcholine may be responsible for its induction

(25, 26)

(27, 28)

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3.3 Time-dependent suppression of insulin release

In addition to acutely regulating insulin secretion, acting as a fail-safe modulator when islets are stimulated with neurohumoral agonists or gut peptides like CCK, and inducing TDP, glucose exerts another additional important effect on the β-cell. When chronically stimulated with high glucose, β-cell insulin secretion fails. Termed the third phase of secretion by Grodsky and coworkers

, β-cell desensitization is an inevitable consequence of sustained high glucose exposure

(6)

(29)

. This process, also referred to as time-dependent suppression (TDS) of release

, may be analogous to the β-cell glucose toxicity noted in human diabetes and other animal models of glucose intolerance

(30-32)

(33)

, glucosamine

. The capacity to induce TDS of insulin secretion is not confined to glucose alone. It can be induced by sustained exposure to agonists as diverse as cholecystokinin

(34)

and forskolin , carbachol

(36)

(35)

. What suppressed islets have in common is their inability to respond, in terms of increases in both PLC-mediated PI hydrolysis and insulin secretion, when subsequently challenged with high glucose

(6, 35, 37)

(29)

and they will respond to an agonist which bypasses the lesion in PLC activation

. It should be emphasized that secretory failure is not the result of insulin depletion since desensitized islets still contain large amounts of insulin

(15, 34)

(30, 38)

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4. GLUCOSE-INDUCED INSULIN SECRETION: ROLE OF PLC ACTIVATION

4.1 Effects of glucose on PLC-mediated phosphoinositide (PI) hydrolysis

The ability of glucose stimulation to increase insulin secretion has been the subject of intense experimental analysis. In large part, this is due to the realization that the emergence of NIDDM is due to β-cell decompensation and their inability to respond to glucose stimulation

. Glucose must be metabolized to augment secretion and the consensus is that the increase in ATP levels or the ATP/ADP ratio results in the closure of ATP-sensitive K^+ channels, cell depolarization and the influx into the β-cell of calcium via voltage-regulated Ca^{2+} channels

(1, 39)

(40-42)

. While glucose-dependent insulin secretion clearly depends on extracellular calcium influx into the β-cell

, this event alone is insufficient to explain the large and rising biphasic insulin secretory response which occurs in response to the hexose. For example, β-cell depolarization with high levels of K^+ and the ensuing sustained increase in intracellular calcium results in a large first phase insulin secretory response with little sustained stimulatory effect on release

(43-45)

(15, 20)

. Other signals dependent on glucose metabolism must be generated and, since the mitochondrial fuels and insulin secretagogues α-ketoisocaproate and monomethylsuccinate duplicate many of the actions of glucose

, a mitochondrial signal in addition to calcium has been postulated

(46)

(45)

. Moreover, mitochondrial signals and calcium are also essential for the full activation of PLC by glucose

leading to the conclusion that the activation of PLC may play an important role in the regulation of glucose-induced insulin secretion.

A variety of techniques have been employed to assess the impact of glucose stimulation on PLC-mediated phosphoinositide hydrolysis. Most investigators incubate islets, usually isolated from rats, in myo-[2-^{3}H]-inositol for various lengths of time. This label is exclusively incorporated into the family of phosphoinositide lipids. After washing to remove unincorporated label, the islets are then stimulated with various agonists and the accumulation of labeled inositol phosphates (IPs), derived from phosphoinositide precursors, serves as the index of PLC activation (Figure 2)

(47, 48)

(14, 42, 49)

. Alternatively islets can be perifused after labeling and the efflux of free [2-^{3}H]-inositol measured in the perifusate along with insulin

. Similar results have been obtained with both approaches and the conclusion reached is that glucose activates PLC, a response dependent upon both glucose metabolism

(45)

(16)

. The precise identity of the metabolic signal has remained elusive but it can be generated by both glycolytic and mitochondrial fuels

and calcium influx

(50, 51)

. like many other tissues

4.2 PLC isozymes in islets and their activation

An additional level of complexity has been added by nature to the activation of PLC. This has to do with the recent demonstration that rat islets

(53)

(52)

express more than one type of PLC. β-cells contain the three major isozymes of PLC (β1, γ1 and δ1) and the available evidence suggests that different classes of insulin secretagogues activate distinct isozymes of PLC

(41, 45)

(52, 54, 55)

. For example, nutrient secretagogues activate the enzyme by increasing intracellular calcium and generating an as yet to be identified mitochondrial signal

. Evidence for this concept derives from the fact that glucose must be metabolized to increase PI hydrolysis, that its effects on PLC activation can be duplicated by both glycolytic and mitochondrial substrates and that the full activation of PLC requires both metabolism and calcium influx into the β-cell. While calcium contributes to the activation of a fuel-regulated PLC, sustained

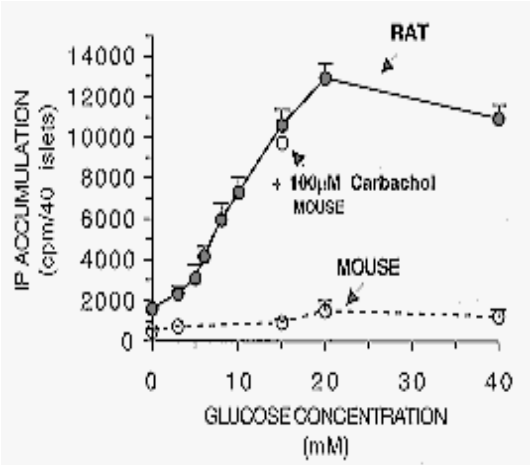

Figure 2. Dose-response Effects of Glucose Stimulation on Phosphoinositide Hydrolysis. Groups of rat (closed circles) or mouse (open circles) islets were incubated for three hr in 3H-inositol, washed to remove unincorporated label and then stimulated with various levels of glucose. The inositol phosphates (IP) accumulating during a 20 minute (rat) or a 30 minute (mouse) stimulatory period with the hexose are shown here plotted against the glucose level. Also indicated is the IP response of mouse islets (open circle) stimulated with the combination of 15mM glucose plus 100μM carbachol demonstrating that with the proper agonist a significant IP response can be generated.

increases in intracellular calcium induced by depolarizing levels of potassium result in only small increments in insulin secretion and IP accumulation

. Furthermore blocking calcium influx reduces but does not abolish glucose-induced IP accumulation

(41)

(45)

. The simplest interpretation of these and other data is that at least two signals--calcium and metabolically-derived factor- -are involved in the activation of the nutrient-regulated PLC.

The concept that neurohumoral agonists such as acetylcholine or its nonhydrolyzable analogue carbachol activate an isozyme of PLC different from that activated by nutrients is supported by both the calcium and metabolic independence of neurohumoral agonists on IP accumulation

(47, 56, 57)

. Most importantly maximal stimulatory levels of glucose and these neurohumoral agonists interact in at least an additive fashion to increase IP accumulation

(54, 57)

. However, perhaps the most cogent data supporting the concept that different isozymes of PLC regulate the responses to fuel and neurohumoral agonists on PLC-mediated PI hydrolysis and insulin secretion comes from studies where the responses of rat islets were compared to those from mouse islets, the two most commonly employed species used to unravel the complex maze of signaling events which regulate insulin secretion

(52)

. Most importantly the divergent effects of glucose on the activation of PLC in these species was paralleled by a marked divergence in the insulin secretory response (Figure 1) to the hexose as well (See below, Section 6).

5. GLUCOSE-INDUCED INSULIN SECRETION: ROLE OF PROTEIN KINASE C ACTIVATION

5.1 Protein kinase C activation in freshly studied islets

The response of the perfused rat pancreas preparation to glucose stimulation is a biphasic release of insulin. In freshly-isolated rat islets, the addition of a stimulatory glucose level (15-20mM) also results in a dramatic and sustained biphasic insulin secretory response

(58, 59)

(See also Figure 1). In particular, the magnitude of the second phase response is 30-40 fold greater than prestimulatory secretion rates. The calcium-dependent activation of PLC also occurs and IP levels, used as a surrogate marker for PLC activation, increase 300-400%

(See Figure 2). Since, in addition to the inositol phosphates, PLC-mediated PI hydrolysis results in the generation of diacylglycerol

(60)

(41, 47)

, the activation of this enzyme might be anticipated as well. In fact, studies in vivo or in vitro with the perfused rat pancreas

, the endogenous activator of PKC

(62)

(61)

or perifused rat islets

(63)

(64)

have demonstrated that PKC translocates from a predominantly cytoplasmic compartment to a membrane one, a response often used to indicate its activation. Furthermore, the phosphorylation state of the MARCKS protein, an established protein target for activated PKC, is increased

. Blocking PKC activation with low levels of the inhibitor staurosporine reduces both MARCKS phosphorylation

(65)

(64)

consistent with an important contributory role for PKC in determining the effects of glucose on the β-cell. PKC activation by glucose or TPA also increases cAMP levels in islets, providing an additional second messenger mechanism to amplify insulin secretion

and second phase secretion from rat islets

(66, 67)

. However, based on studies with cultured islets, the conclusion has been reached that PKC activation may not be involved in glucose-induced insulin secretion (68, 69). The reasons for this continuing controversy and a possible resolution to this issue deserve further comment.

5.2 PKC Activation and Glucose-induced Insulin Secretion from Cultured Rat Islets

In a series of early studies using the phorbol ester TPA, an exogenous PKC activator, to stimulate the β-cell we suggested that the activation of PKC may be playing an important role in the rising second phase secretory response evoked by glucose (70)

. We based this suggestion on the slowly rising and sustained release of insulin that followed β-cell exposure to the phorbol ester

(67, 70)

, a response that qualitatively was somewhat reminiscent to that seen with glucose stimulation. This concept- that PKC activation regulated at least in part glucose-

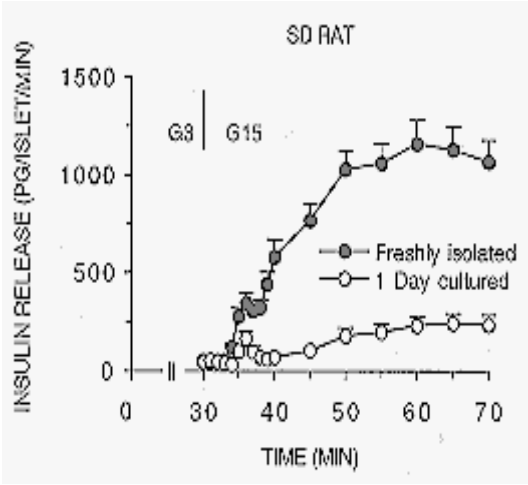

Figure 3. Glucose-induced Insulin Secretion is Impaired from Rat Islets after Short Term Culture. Two groups of rat islets were studied. One group (closed circles) was perifused with 3mM glucose (G3) for 30 min and with 15mM glucose (G15) immediately after isolation. The second group (open circles) was cultured 22-24 hours in CMRL-1066 medium supplemented with 10% fetal calf serum prior to perifusion. The glucose level of the medium was 5-5.5mM. Note the dramatic reduction in secretion from cultured islets.

induced insulin secretion-has been both challenged and supported. Most of the negative data concerning the role of PKC activation has been generated with islets cultured for various periods of time after isolation. However, the activation of PKC is not the only aberration cultured islets demonstrate. When stimulated by high glucose, the accumulation of IPs in one-day cultured islets is reduced

, high glucose fails to translocate PKC to the membrane

(68)

(71)

, islet content of PLCδ1 falls by about 50% (unpublished observation), residual insulin release is immune to inhibition by staurosporine

and, when primed by high glucose, time-dependent potentiation is not induced (unpublished observation). Most telling, however, is the minimal insulin secretory response observed when cultured rat islets are stimulated with glucose

(72)

(68, 71)

. The magnitude of this response, 4-5 fold above basal stimulatory rates (See Figure 3), stands in contrast to the robust 30-40 fold increase seen from the perfused pancreas preparation or from our freshly-isolated rat islets. The decline in islet responsiveness to glucose observed after culturing is not confined to rat islets. Cultured human islets suffer the same functional decline. For example the human β-cell response to glucose stimulation in vivo is a 10-20 fold increase in second phase insulin secretion rates

. In sharp contrast the second phase insulin secretory response to 28mM glucose from cultured human islets is flat

(72)

(10,
12)

, less than 2-fold greater than prestimulatory secretion rates and is paralleled by the minimal activation of PKC as well

(72)

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5.3 Insulin secretion from PKC-depleted rat islets

In an attempt to establish the involvement of PKC activation in the sequence of biochemical events which regulate physiologic glucose-induced insulin secretion from rat β-cells, several groups have cultured islets in the presence of TPA to deplete the islet of PKC. There are actually at least three issues here but only one is usually addressed. First is the effect of culturing which alone impairs glucose-induced insulin secretion

(71, 73, 74)

irrelevant of any other additions to the culture medium. The second is the added effect of sustained PKC stimulation on an islet whose secretory integrity is deteriorating as a result of culturing. Finally, the tacit assumption that only PKC content is altered and that all of the protein substrates for PKC remain in a basal or unstimulated state is unwarranted. Since PKC activation exerts long term effects on β-cell sensitivity, effects presumably mediated by the sustained phosphorylation of PKC substrates

(75)

, the fact that PKC is depleted does not address the possible contribution of these phosphoproteins to insulin release

(76)

. To emphasize the problem with cultured islets one only has to study the secretory data from one report (69) often cited as evidence that PKC activation is not involved in the physiologic regulation of glucose-induced insulin secretion. In this study, the insulin secretory response after 20-24 hour of culture from control islets stimulated with 5.6mM glucose was 0.44 ng/h per islet. The insulin secretory response of cultured control islets to 20mM glucose increased to 0.75 ng/h per islet and less than a doubling of insulin output occurred. This observation stands in sharp contrast to the 30-fold increase in release rates we and others have obtained with freshly isolated islets and casts a considerable doubt on the physiologic significance of any further observations made with such profoundly impaired islets irrelevant of their PKC content.

To conclude that PLC/PKC activation is not involved in the physiologic regulation of insulin secretion from a preparation (cultured islets) which differs in several substantial ways from the responses of fresh islets is inappropriate. We have repeated many of these studies with cultured rat islets and arrived at the same conclusion

(71) (54, 55)

. In islets whose insulin secretory responsiveness to glucose is dramatically impaired by culturing, the activation of PLC/PKC signaling events may not be involved in the minimal insulin secretory responses observed. We and others

About 16 years ago both Lenzen (39) and Berglund (77) have dealt with this issue in several articles. However, studies with cultured islets have reinforced our conviction that information flow in the PLC/PKC signaling system is an essential component of physiologic glucose-induced insulin secretion.

6. SPECIES DIFFERENCES IN PLC ACTIVATION, GLUCOSE-INDUCED INSULIN SECRETION, TIME-DEPENDENT POTENTIATION AND TIME DEPENDENT SUPPRESSION

6.1 Glucose-induced insulin release from mouse islets

(78)

(7, 8, 79)

reported that the insulin secretory responses of the perfused mouse pancreas preparation to glucose stimulation differed significantly from those seen with the perfused rat pancreas preparation studied under comparable conditions. While acute first phase release rates were similar, the mouse β-cell response to glucose stimulation was notable for the absence of a rising second phase secretory response. For example, when rat β-cells were stimulated with high glucose, 30-40 fold increments in insulin release rates were observed with the perfused pancreas preparation

or from freshly-studied perifused islets

(58, 65)

(52)

(See Figure 1). Second phase responses from mouse islets are flat and from a quantitative perspective much smaller (2-4 fold above prestimulatory rates) than those observed from rat islets

. This deviation in second phase release from mouse β-cells has recently been confirmed by Grodsky and coworkers

(52)

(80)

and by Sharp and coworkers

, by our group using perifused mouse islets

(81)

studying release from βHC-9 mouse tumoral cells . This difference in glucose sensitivity is unrelated to islet insulin content

(80)

. Of particular importance, the deviation in mouse islet responsiveness to glucose also stands in contrast to the large and rising second phase responses from human islets studied in vivo with the hyperglycemic clamp technique

(10, 12, 82)

. Thus, while rat and human islets respond to sustained glucose stimulation with a large and rising second phase insulin secretory response, mouse β-cells fail to demonstrate this response to glucose.

6.2 PLC activation in mouse islets

In an attempt to explain the dichotomy in the insulin secretory responses to glucose stimulation which exist between rat and human islets on the one hand and mouse islets on the other, we first focused our attention on information flow in the PLC/PKC pathway. Detailed glucose dose-response curves looking at the accumulation of inositol phosphates, a surrogate and highly sensitive marker for PLC activation, revealed a possible biochemical explanation for the anomalous behavior of mouse islets when stimulated by glucose. In response to the hexose, minimal IP accumulation was observed (Figure 2). This suggested to us that at least part of the explanation for the smaller secretory effect of glucose was attributable to the minimal generation of PI-derived signaling molecules. Since we knew from our rat islet studies that carbachol activated an isozyme of PLC different from the one activated by glucose, we next measured the IP responses to this agonist. Comparable stimulatory effects were noted

(54)

and, most importantly, the addition of carbachol or the protein kinase activator TPA together with 15mM glucose resulted in the emergence of a brisk biphasic insulin secretory response from mouse islets (Figure 4). Before concluding this section it might be posited that the failure of glucose to stimulate PLC to the same quantitative extent in mouse islets as in rat, and presumably human islets, would leave the animal at risk for hyperglycemia. It must be remembered however that food consumption in these animals is accompanied by an increase in vagal tone and CCK secretion. These factors provide the necessary PLC-generated signals to support insulin secretion. What makes human and rat islets so different and unique is that they have a backup or supplemental system in that, in addition to neural or incretin factors, fuel or nutrient molecules like glucose are also capable of providing significant PLC-generated second messengers to reinforce the secretory response.

6.3 Time-dependent potentiation in mouse islets

The failure of glucose to evoke a rising second phase insulin secretory response from mouse islets when compared to the responses of human or rat islets studied under comparable stimulatory conditions is not the only anomaly reported to exist between these species. In follow-up studies Berglund

(7, 16)

(83)

and human islets

reported that prior high glucose stimulation of mouse β-cells failed to prime or sensitize them to restimulation. This phenomenon, also referred to as time-dependent potentiation (TDP), can be induced in both rat

(84, 85)

(83)

by prior short term glucose exposure. In what may turn out to be a particularly prescient remark, Berglund

concluded that the failure of glucose to induce TDP in mouse β-cells may be related to the failure of glucose to induce a rising second phase secretory response in this species. The fact that the magnitude of the second phase response may actually regulate the priming effect of glucose was initially suggested by Grodsky

(4)

(29, 70, 86, 87)

25 years ago. The further characterization of the factor(s) which limit glucose-induced insulin secretion from mouse β-cells and accounts for the anomalous behavior of mouse β-cells to glucose stimulation when compared to human or rat β-cells may offer a most unique opportunity to elucidate the factors which regulate glucose-induced insulin secretion. Because of our interest in this process and because of our earlier suggestion that both the rising second phase response and the induction of TDP seen when rat islets are stimulated with glucose may be dependent on events associated with PLC/PKC activation

, we explored these differences in more detail and attempted to explain these species-dependent response patterns to glucose stimulation.

Confirming the findings made with the perfused pancreas preparation, mouse islets are immune to the sensitizing effect of prior short term glucose exposure. When briefly exposed to glucose, rat islets

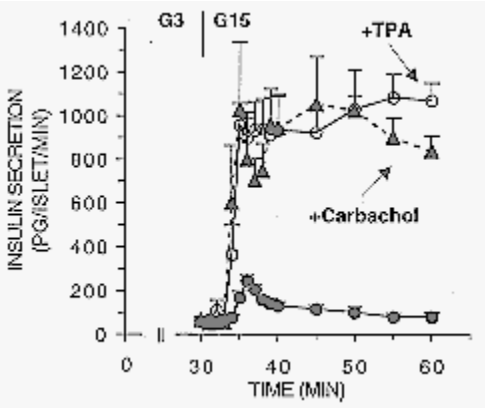

Figure 4. Stimulation of Rising Second Phase Insulin Secretory Responses from Mouse Islets. Groups of islets were isolated from CD-1 mice and perifused with 3mM glucose (G3) for 30 min. At this time one group was stimulated with 15mM glucose (G15) alone (closed circles), a second group with 15mM glucose plus 500nM of the phorbol ester tetradecanoyl phorbol acetate (TPA, open circles), and the third group was stimulated with 15mM glucose plus 100 µM carbachol (closed triangles). Note that while glucose alone is without any effect in stimulating a rising second phase, the addition of carbachol or TPA together with 15mM glucose evokes a large, rising second phase response.

respond to subsequent restimulation with a dramatically enhanced first phase insulin secretory response

(88)

(See Figure 5) Mouse islets could not be sensitized under conditions where rat islets so readily exhibit this response pattern. If, as we have suggested, the failure of glucose to activate PLC in mouse islets is the immediate cause of the failure of the hexose to induce priming, then it might be suggested that carbachol or TPA should be able to induce TDP. This was verified is subsequent studies

(88)

(See Figure 6) and further supported the concept that the species differences in the expression and activation of PLC played a major and determining role in the species-dependent response patterns to glucose stimulation.

6.4 Glucose fails to induce time-dependent suppression (TDS) in mouse islets

While the positive stimulatory actions of glucose on the β-cell have been the primary foci up until now, it is also clear that sustained exposure to the hexose impairs insulin secretion. Termed TDS, it can also be induced by other agonists including but not confined to those compounds which act initially to increase information flow in the PLC/PKC signaling system. Thus, TDS can be induced by long term (2-3 hour) exposure to cholecystokinin (CCK) or to carbachol as well as by sustained exposure to forskolin, which elevates cAMP to supraphysiological levels in islets (89). In islets suppressed by any one of these compounds, the common biochemical lesion and the one which appears to account for reduced insulin secretion is the impaired capacity of subsequent restimulation with glucose to activate PLC. Our working hypothesis to account for the induction of TDS is that the sustained activation of PKC or PKA results in the phosphorylation of PLC, a type of negative feedback and an event which reduces PLC's ability to subsequently respond to stimulation. This proposed model of inhibition is consistent with what has been observed in other systems as well: PKC activation inhibits PLC activation (53, 90, 91)

. Moreover, PLC activation is also inhibitable by cAMP as well, providing an explanation for the inhibitory action of forskolin on β-cell PLC and its capacity to induce TDS of insulin release. If sustained PLC/PKC activation by high glucose is responsible for the induction of TDS of secretion in rat islets, then mouse islets which respond poorly in terms of PLC-mediated PI hydrolysis should be relatively immune to the desensitizing effect of the hexose. This has been confirmed using perifused mouse islets previously exposed to 20mM glucose for 3 hr (See Figure 7). For example, 3 hour exposure of rat islets to 20mM glucose impaired the capacity of these islets to respond subsequently to stimulation with 20mM glucose plus 100μM carbachol; 20mM glucose induced TDS of release. Mouse islets secretory responses studied after a 3 hour incubation period with 20mM glucose were not suppressed when stimulated with 20mM glucose plus 100μM carbachol; 20mM glucose failed to induce TDS of release in mouse islets. Studies with mouse islets have afforded us a most unique opportunity to dissect out the contribution of glucose signaling via the PLC/PKC cascade to the regulation of insulin secretion.

7. THE BETA CELL, OBESITY AND NIDDM

Before summation it is perhaps not unreasonable to place the basic science studies discussed above into some type of clinical perspective. The question might be posed as to how signaling in the PLC/PKC system may be involved in altered patterns of insulin secretion established to occur in both obesity and NIDDM. In obesity, β-cell secretion is dramatically amplified. Thus, in both human obesity

(55)

(92, 93)

(94, 95)

and in several well characterized animal models of obesity

, early and perhaps even primary changes in β-cell sensitivity to stimulation have been observed. In fact, even in preobese mice, the sensitivity of their islets to agonists which increase information flow in the PLC/PKC signaling system is enhanced and contributes to the early and perhaps primary hyperinsulinemia in these animals

(96)

(97-99)

. Since insulin resistance can be acquired as a consequence of hyperinsulinemia

, changes at the level of β-cell signal transduction systems which amplify their stimulatory effect on insulin release, may be primary etiologic components in the odyssey to obesity

(100)

. Finally, while there is little doubt that β-cell failure

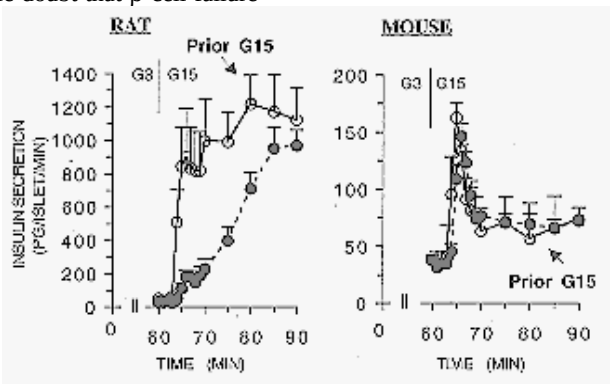

Figure 5. Glucose Stimulation Induces Time-Dependent Potentiation in Rat but not Mouse Islets. Groups of rat (left, open circles) or mouse (right, open circles) islets were perifused for 30 min with 3mM glucose (G3) followed by 15 min with 15mM glucose (G15). After a 15 min washout with 3mM glucose they were stimulated with 15mM glucose for 30 min and this is the period shown. The responses of control islets (closed circles) perifused for 60 min with 3mM glucose prior to 15mM glucose stimulation are also shown. Note that prior glucose sensitized rat but not mouse islets. Note also the change in scale between panels.

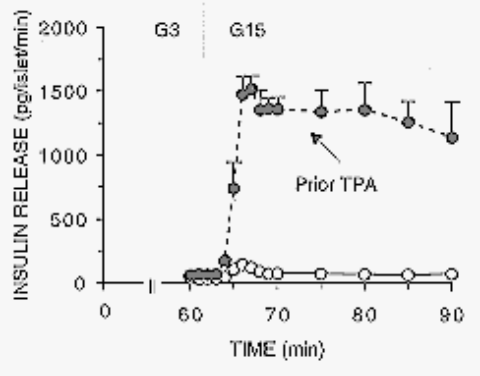

Figure 6. Prior Exposure to the Phorbol Ester TPA Induces Time-Dependent Potentiation in Mouse Islets. Two groups of mouse islets were studied. One group (open circles) was perifused for 60 min with 3mM glucose (G3) prior to stimulation with 15mM glucose (G15). The second group (closed circles) was perifused for 30 min with 3mM glucose, 15 min with 3mM glucose plus 500nM TPA and for an additional 15 min with 3mM glucose alone. They were then stimulated with 15mM glucose and this is the period shown here.

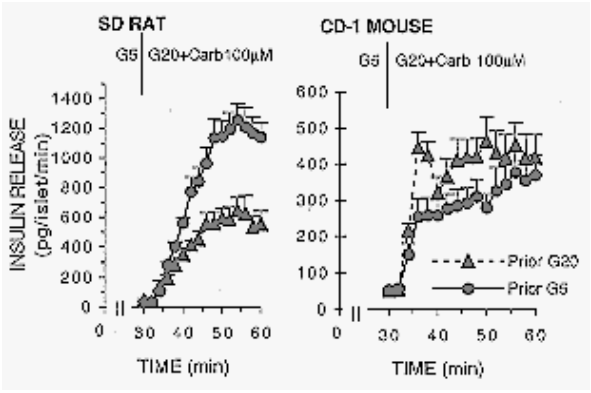

Figure 7. Effects of Prior 3hr. Exposure to 20mM Glucose on Insulin Release Rates from Rat or Mouse Islets: High Glucose Fails to Induce Suppression in Mouse Islets. Groups of islets were isolated from Sprague-Dawley (SD) rats (left) or CD-1 mice (right) and incubated for 3 hr in 5mM glucose alone (closed circles, solid line) or 20mM glucose alone (closed triangles, dashed line). All groups of islets were then perifused for 30 min with 5mM glucose (G5) and for an additional 30 min with the combination of 20mM glucose (G20) plus 100µM carbachol (Carb). This combination of agonists was used because glucose alone is such a poor stimulant for secretion from mouse islets. Note the reduction in release from rat islets due to prior 20mM glucose exposure but the slight increase from mouse islets.

or decompensation is essential in the development of frank NIDDM, the nature of the biochemical lesion responsible for the reduction in insulin secretion is not known. However, since β-cell failure to secrete enough insulin to maintain glucose homeostasis is a consequence of hyperglycemia

(32)

and since high glucose desensitizes the β-cell by inhibiting PLC activation (33, 101), a reasonable suggestion is that the impaired activation of β-cell PLC may precipitate NIDDM in some individuals.

9. SUMMARY AND PERSPECTIVE

Because of the essential role of insulin in the maintenance of fuel homeostasis, the role of the signal transduction systems which regulate β-cell secretion of the hormone assume physiologic and clinical significance. In the present review, we have attempted to draw the readers attention to the key and essential role of information flow in the PLC/PKC signal transduction pathway in determining how the β-cell works and why it may fail or decompensate. Results from studies support the concept that this system is involved in glucose-induced biphasic insulin secretion, in particular the rising second phase response, the induction of TDP, characterized by a markedly enhanced first phase response, and the induction of TDS where insulin secretory failure occurs. Studies from several labs have demonstrated marked species differences between rat and mouse β-cell sensitivity to glucose stimulation, differences we have attributed to a dichotomy in the information flow in the PLC/PKC signaling system. Finally, the potential role of this transduction system in the etiology of hyperinsulinemia, insulin resistance, obesity and, all too often, in NIDDM has been suggested. Along these same lines, it would be of particular interest to ascertain how overexpression of PKC influences glucose homeostasis and whether treating mouse islets with viral vectors containing the cDNAs for the various isozymes of PLC, as has been done with other enzymes

(102)

, converts their response patterns to those reminiscent of rat islets. Finally, in attempts to genetically engineer pancreatic β-cells with an eye toward their transplantation, consideration will have to be given to the content and expression of the PLC isozymes and PKC in these cells if the physiologic regulation of insulin secretion is to be duplicated.

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