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The role of cyclic AMP in insulin release

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1. Introduction

The idea that adenosine-3′,5′-cyclic monophosphate (cyclic AMP) may act in the pancreatic B-cell as a second messenger was probably first raised when Samols et al.[72] reported that glucagon stimulates insulin release in man. The inhibitory effect of catecholamines on insulin secretion, first described by Coore and Randle[17], was also soon considered within the framework of this idea. Cerasi and Luft[11] even postulated that glucose, in addition to serving as a metabolizable substrate, acted in the B-cell on a specific membrane receptor leading to activation of adenylate cyclase, increased cyclic AMP production and stimulation of insulin release. In the latter model, cyclic AMP was considered to represent the signal for insulin release, whereas the metabolism of glucose would merely modulate this signal function, for example by increasing ATP availability to adenylate cyclase. At variance with the latter view, we had defended the concept that cyclic AMP should not be considered as a signal for insulin release but, instead, as a modulator of the metabolic and secretory response to nutrient secretagogues[53].

Almost 20 years have elapsed since cyclic AMP entered the field of insulin release, and more than a dozen years have passed since the contrasting views expressed above were introduced in relevant textbooks. In retrospect, it appears that the role of cyclic AMP in insulin release merits reevaluation. In the present report, the enzymes involved in the regulation of cyclic AMP synthesis and breakdown, the target systems responsive to this nucleotide and the role of cyclic AMP in the regulation of insulin release will be successively taken into consideration.

2. Cyclic AMP synthesis and breakdown

In the islets like in other tissues, the cell content in cyclic AMP is thought to reflect the balance between its rate of synthesis, breakdown and release in the extracellular fluid.

a) Cyclic AMP synthesis: adenylate cyclase

As judged by cytochemical criteria, adenylate cyclase is localized in the B-cell exclusively and almost uniformly in the plasma membrane[34]. In a particulate fraction (12,000 × g × 20 min) derived from the islets, the enzyme displays a Km for ATP close to 0.07 mM, whether in the presence or absence of NaF (10 mM)[49]. The temperature dependency (Arrhenius' plot) yields an apparent energy activation of 8.0 and 18.4 kcal/mole in the absence and presence of NaF, respectively[44]. The basal value for adenylate cyclase, expressed per islet-equivalent, is much higher in islet crude homogenates than in a subcellular fraction[21]. This difference is not solely attributable to uncomplete recovery of the enzyme in the particulate fraction. It may be due, in part, to be presence in the cytosol of islet cells of an unidentified phosphocompound which doubled the activity of adenylate cyclase in a particulate fraction (27,000 × g × 20 min) derived from mouse islets[88]. This factor was found to be dialysable, resistant to heat, sensitive to charcoal treatment and alkaline phosphatase, insensitive to digestion with trypsin; and distinct from either GTP, NAD or phospho-enol-pyruvate.

Consideration on the modulation of adenylate cyclase activity in the acellular system will here be restricted to those findings which are most relevant to the overall theme of this review and are not examined in greater detail elsewhere in this report.

Ca^{2+} (0.1 to 0.7 mM or more) causes a dose-related inhibition of adenylate cyclase activity[94]. However, in the presence of Ca^{2+}, the enzyme is activated by calmodulin[80,94]. At a fixed concentration of calmodulin, the threshold Ca^{2+} concentration for activation of the enzyme is close to 10^{-7} M, with an apparent Ka close to 10^{-5} M[94]. At a fixed concentration of Ca^{2+} (0.2 mM), the apparent Ka for calmodulin is close to 0.1 μM[93]. Curiously, Thams et al.[87] were unable to detect activation of adenylate cyclase by Ca-calmodulin in a mouse, as distinct from rat, islet particulate fraction.

Most investigators failed to detect any direct effect of

glucose (or other nutrient secretagogues) upon adenylate cyclase activity in islet homogenates[33,42,88,94]. In one report[8], glucose (17 mM), phospho-enol-pyruvate (10 μM) and pyruvate (10 μM) were claimed to activate islet adenylate cyclase, but this could not be confirmed when the composition of the assay medium was modified and the authors felt that no firm conclusion could be reached concerning the effect of glucose itself or its metabolites on adenylate cyclase activity[87].

b) Cyclic AMP breakdown: phosphodiesterase

Cyclic AMP phosphodiesterase activity in insular material has been characterized by different investigations[22,42,71,73]. There appears to be two enzyme systems with Km values for cAMP of 2–9 μM and 30 μM or more, respectively[4,22,73]. Optimal pH is in the range of 8.2–8.5[4,73]. The apparent molecular weight of the enzyme is 200,000 kDa[73]. About 70% of the total insular activity is found in the postmicrosomal supernatant, particulate activity also being present[71,73].

Most studies on the regulation of phosphodiesterase deal with the low Km enzyme. Most investigators agree that the enzyme is, as expected from studies in other tissues, inhibited by theophylline, caffeine, 3-isobutyl-1-methylxanthine, sulfonylurea (glibenclamide, chlorpropamide, tolbutamide), and activated by imidazole. Glucose and glucose-6-phosphate do not seem to affect the activity of phosphodiesterase. Calmodulin may exert a modest activation of islet phosphodiesterase[47,83], as reviewed elsewhere in this volume[92].

c) Cyclic AMP distribution in and outflow from islet cells

Leitner et al.[45] reported that, in islets prelabelled with [2-^{3}H]adenine, secretory granules contain 5% of the total amount of tritiated adenine nucleotides present in the islet cells and that labelled cyclic AMP represents 14–18% of the [^{3}H]adenine nucleotides associated with secretory granules. Since the latter ratio is decreased to about 2% in the crude homogenate, it would appear that about 40% of the total amount of tritiated cyclic AMP present in the islets is associated with secretory granules. This remains to be confirmed.

Several studies have indicated that a fraction of the cyclic AMP generated in the islet cells is extruded in the extracellular fluid[7,25,60,91]. This release of cyclic AMP occurs independently of and can be dissociated from the release of insulin[7]. It is inhibited by papaverine (0.1 mM) or probenecid (1.0 mM)[7]. Over 30 min incubation in the presence of D-glucose (20 mM) or 3-isobutyl-1-methylxanthine (1.0 mM), the release of cyclic AMP amounts to at least 10–20% of the intracellular content measured at the end of incubation[7]. In one series of experiments performed over 60 min incubation, the amount of cyclic AMP released by the islets was even twice higher than the final islet content[25]. The same authors, however, reported a much lower fractional release of tritiated cyclic AMP from islets prelabelled with [2-^{3}H]adenosine, in which case the release of the nucleotide over 60 min incubation did not exceed 50% of the final islet content[60].

3. Mode of action of cyclic AMP

a) Stimulation of protein kinase

Cyclic AMP-responsive protein kinase activity was identified by several authors in islet homogenates[19,20,27,35,63,64,84]. This topic is considered in detail elsewhere in this volume[28]. It seems reasonable to assume, but remains to be proved, that several of the functional effects of cyclic AMP to be discussed below are linked to the phosphorylation of suitable proteins.

b) Metabolic and biosynthetic effects

The bulk of the evidence suggests that, especially at high glucose concentrations, the enhancing action of cyclic AMP upon insulin release is not attributable to any marked facilitation of nutrient catabolism in the islet cells. A modest effect of cyclic AMP upon metabolic variables should, however, not be ruled out.

For instance, it was shown that agents raising the islet content in cyclic AMP stimulate glycogenolysis in glycogen-rich islets[36]. Moreover, even in normal islets which do not contain any significant store of glycogen, there are indications that a rise in the cyclic AMP content of the islet cells may cause a modest metabolic response. Thus, theophylline may increase the ATP/ADP ratio and adenylate charge of islets deprived of exogenous nutrient[41]. This coincides with a modest increase in the output of $^{14}CO_2$ from islets prelabelled with [U-^{14}C]palmitate and incubated in the absence of exogenous nutrient[41], and an increase in lactate production best seen at low glucose concentrations or in islets removed from fasted rats[46,78]. Theophylline, however, does not affect the production of $^{14}CO_2$ from islets prelabelled over 30 min preincubation in the presence of L-[U-^{14}C]-glutamine (1.0 mM) and incubated for 30 min in the absence of exogenous nutrient. Thus, relative to the ^{14}C content of the prelabelled islets, the output of $^{14}CO_2$ during incubation averaged 13.1 ± 0.7 and 14.9 ± 0.5% ($n = 9$) in the absence or presence of theophylline (1.4 mM), respectively, as compared to a 'blank' value of 5.1 ± 0.3% ($n = 8$) in the presence of antimycin A and rotenone (10 μM each). It remains to be investigated, therefore, to which extent the metabolic effects of theophylline could reflect stimulated lipolysis and/or protein catabolism. These effects could account for the capacity of theophylline (or dibutyryl-cyclic AMP) a) to provoke stimulation of proinsulin biosynthesis, preferentially relative to that of other islet peptides, in islets incubated at a low concentration of glucose[55,60], and b) to cause a modest decrease in the fractional outflow rate of ^{86}Rb from prelabelled islets perifused in the absence of glucose[9]. It should be noted, however, that, at variance with the situation found in response to nutrient secretagogues, theophylline fails to provoke any increase in the output of radioactivity from islets prelabelled with ^{32}P-orthophosphate[10] and perifused in the presence of 4.2 mM glucose.

At high glucose concentrations (e.g. 16.7 mM), and in islets removed from fed rats, neither theophylline nor dibutyryl-cyclic AMP exert any significant effect upon glucose uptake, utilization and oxidation, lactate output or proinsulin biosynthesis (relative to that of other islet

peptides)[55,78]. Yet, it is as such high glucose concentrations that these agents exert their most marked enhancing action upon insulin release. This strongly suggests that the major site of action of cyclic AMP in the secretory sequence is distal to the site of nutrient identification.

c) Effect on Ca^{2+} fluxes in islet cells

In the first study devoted to the effect of cyclic AMP upon ^{45}Ca net uptake by islets, a modest positive response was only observed at a glucose concentration close to the threshold value for stimulation of insulin release[6]. Such a positive response was not observed at higher glucose concentration, at which the enhancing action of cyclic AMP upon insulin release is most obvious. Hence, it was concluded that the major mode of action of cyclic AMP did not consist in the stimulation of Ca^{2+} uptake.

A series of recent studies by Henquin and his coworkers suggest nevertheless that a stimulant action of cyclic AMP upon Ca^{2+} inflow into the B-cell should not be overlooked[30,31]. For instance, over 25 min incubation in the absence or presence of dibutyryl-cyclic AMP (1.0 mM), the uptake of ^{45}Ca (measured during the last 5 min of incubation) was modestly (+17 to 38% increment) but significantly increased by the cyclic nucleotide in the range of glucose concentration between 7.0 and 8.0 mM and at Ca^{2+} concentrations between 1.0 and 2.5 mM[30].

Likewise, forskolin (5 μM) or dibutyryl-cyclic AMP (1.0 mM) increase electrical activity in mouse pancreatic B-cells exposed to glucose 7–10 mM (but not 3 mM)[30,31]. The response to forskolin is more rapid than that to dibutyryl-cyclic AMP. Cobalt (1.0–2.5 mM) suppresses glucose-stimulated electrical activity even in the presence of forskolin or dibutyryl-cyclic AMP. From these results, Henquin concluded that cyclic AMP may modulate the permeability of Ca channels in the plasma membrane and, hence, facilitate Ca^{2+} influx in insulin-secreting cells. It could be questioned, however, whether an increase in electrical activity is necessarily synonymous of a facilitation of Ca^{2+} influx. The recent observation that tumor-promoting phorbol esters, which fail to affect the net uptake of ^{45}Ca[56], also promote electrical activity in islet cells[68] could be quoted against the latter equation. Incidentally, in addition to increasing bioelectrical activity, Henquin et al.[32] also recently reported that forskolin (0.2 μM) suppresses the slow cyclic variations (periodicity about 4–5 min) of bioelectrical activity seen in about 45% of mouse B-cells exposed to glucose in the 10–15 mM range.

It was proposed by Brisson et al.[5,6] that cyclic AMP may act in the secretory sequence by causing an intracellular redistribution of Ca^{2+}, to favor the cytosolic accumulation of this cation, rather than by stimulating the net uptake of Ca^{2+} by the islet cells. Such a proposal is supported by radioisotopic data suggesting that a) a rise in cyclic AMP may cause the moblization of ^{45}Ca from intracellular stores in prelabelled intact islets[6,81], b) the nucleotide may inhibit the uptake of ^{45}Ca of subcellular preparations isolated from the islets[77], and c) the subcellular distribution of ^{45}Ca in intact islets is indeed affected by cyclic AMP[26]. In addition to these radioisotopic data, it was also observed that forskolin, theophylline or dibutyryl-cyclic AMP are able to restore, to a limited extent, the secretory response to glucose in islets incubated at a low concentration of extracellular Ca^{2+}[6,49]. In the case of forskolin, such a partial restoration was even obseved in islets incubated in the absence of Ca^{2+} and presence of EGTA[49].

d) Response of the effector system to Ca^{2+}

It is conceivable that cyclic AMP acts, in part at least, independently of or in concert with Ca^{2+} to enhance insulin release. For instance, cyclic AMP might augment the responsiveness to Ca^{2+} of the effector system which controls the access of secretory granules to their site of exocytosis at the plasma membrane. The experimental validation of such a concept will depend on the measurement of cytosolic ionized Ca^{2+} concentration in islets exposed to such agents as forskolin. Meanwhile, it is worthwhile to note that a dual mode of action of cyclic AMP to both decrease the fractional removal rate of cytosolic Ca^{2+} by the vacuolar system and to augment the secretory response to a given concentration of cytosolic Ca^{2+} was postulated in order to simulate the fluxes of ^{45}Ca, production of cyclic AMP and release of insulin in a mathematical modelling of cyclic AMP-Ca^{2+} interactions in pancreatic islets[59]. Incidentally, the effect of cyclic AMP on intracellular Ca^{2+} distribution could also be simulated by postulating that the nucleotide increases the fractional outflow rate of Ca^{2+} from the vacuolar pool[76]. It was even considered that such an increase may participate in the process of Ca-stimulated Ca^{2+} release by the vacuolar system[75]. The latter process was introduced in the mathematical model to account for the stimulation of ^{45}Ca outflow which is observed in prelabelled islets when the cytosolic concentration of Ca^{2+} is increased as a result of facilitated $^{40}Ca^{2+}$ inflow into the islet cells[75].

e) Facilitation of insulin release

Whatever its precise cytophysiological determinants, it is well established that a rise in the cyclic AMP content of islet cells results in the facilitation of insulin release. There is no unanimity among investigators as to the modulation of the secretory response to agents raising islet cell cyclic AMP content (e.g. glucagon, 3-isobutyl-1-methylxanthine, theophylline, dibutyryl-cyclic AMP, forskolin) by the ambient glucose concentration. According to some authors, these agents are potent stimulators of insulin release at low glucose concentrations. For instance, Steinberg et al.[82] recently reported that, either over 10 or 30 min incubation, 3-isobutyl-1-methylxanthine (0.4 mM), glucagon (10 μM) or forskolin (20 μM) stimulate insulin release in the presence of 1.7 mM glucose to the same high secretory rate as observed in the sole presence of glucose 16.7 mM! Yet, in two other recent studies, forskolin (30–50 μM) failed to affect insulin release, whether over 3 or 90 min incubation, from islets exposed to 2.0–2.8 mM glucose[49,90].

From a careful examination of the extensive literature on this crucial matter, but inevitably biased by the data

collected in our laboratory, we feel obliged to define the relationship between the secretory response to cyclic AMP and glucose concentration as follows.

Although a minor and/or transient stimulation of insulin release by agents thought to specifically increase the cyclic AMP level in islet cells was occasionally claimed to be detectable in islets deprived of exogenous nutrient and not exposed to any non-nutrient secretagogue, there is now general agreement on the concept that cyclic AMP only causes obvious and sustained stimulation of insulin release when the islets are exposed to suitable secretagogues. For instance, when glucose is present at a concentration close to or in excess of the threshold value required for stimulation of insulin release, the enhancing action of cyclic AMP analogs, glucagon, forskolin, cholera toxin or phosphodiesterase inhibitors upon insulin release is fairly evident, being usually most marked, in absolute terms, at high glucose concentrations. There is, however, at least one exception to this behavior, i.e. when the islets contain large amounts of glycogen, in which case insulin release can be stimulated by theophylline in the absence of exogenous glucose[53, 57].

4. Role of cyclic AMP in the control of insulin release

The last topic in this report concerns the role that cyclic AMP may play in the physiological control of insulin release. Two distinct aspects of this problem will here be taken into consideration, namely the participation of cyclic AMP in the normal process of glucose-induced insulin release and its role in the hormonal modulation of B-cell secretory activity.

a) Role of cyclic AMP in glucose-induced insulin release

As already mentioned, there is an ample body of evidence indicating that a rise in the cellular content of cyclic AMP, even if much more marked than that evoked by glucose in high concentrations, is not sufficient to cause any sizeable increase in insulin output when the islets are exposed to a concentration of the sugar (e.g. 2.8 mM) sufficient to both maintain a normal ATP content in the islet cells[50] and to enhance insulin release evoked by certain nonnutrient secretagogues[79]. This strongly suggests that cyclic AMP cannot be considered as the essential second messenger mediating the insulinotropic action of glucose.

It is also well documented that, in intact islets, glucose causes a modest increase in cyclic AMP production which, however, does not exceed twice the basal value. This effect of glucose is most evident in absolute, but not in relative, terms in the presence of phosphodiesterase inhibitors. In the absence of the latter agents the effect of glucose is, in absolute terms, so modest that it failed to be detected by several investigators[16, 61, 62]. Most studies suggest that the time course for the glucose-induced increase in the cyclic AMP content of the islet is a rapid phenomenon detected within 2–3 min of stimulation, but reaching its peak value after only 15–30 min[3, 12, 25, 70, 91]. To our knowledge, an early biphasic pattern for the glucose-induced increase in cyclic AMP content, analogous to that characterizing the pattern of insulin release, was only documented in one study[97].

The modality by which glucose increases cyclic AMP production will now be considered. In most studies, no effect of glucose itself upon adenylate cyclase activity could be detected in islet cell homogenates or subcellular fraction (see above). Since islet adenylate cyclase is activated by Ca-calmodulin, Valverde et al. proposed that the effect of glucose to increase cyclic AMP in intact islets could be mediated by Ca-calmodulin and, hence, be secondary to an increase in cytosolic Ca^{2+} concentration[94]. The latter view is supported by the finding that the stimulant action of several nutrient secretagogues upon cyclic AMP production by intact islets is suppressed when the islets are incubated in the absence of extracellular Ca^{2+} [91, 97].

The fact that glucose increases cyclic AMP production in intact islets and the fact that cyclic AMP enhances glucose-stimulated insulin release would suggest that, in the normal process of glucose-induced insulin secretion, cyclic AMP may play a role as an endogenous enhancing factor. It is also conceivable that a minimal, permissive, rate of cyclic AMP formation is required for maintenance of a normal secretory response to glucose. Little factual evidence is available to document these concepts. The inhibition of glucose-induced insulin release by either calmodulin antagonists (e.g. trifluoperazine) used to prevent the glucose-induced activation of adenylate cyclase[92], or phosphodiesterase activators (e.g. imidazole), used to accelerate cyclic AMP breakdown[51, 52], would support such concepts, if these agents had unambiguously been shown to act as site-specific inhibitors. This is not the case, however. Indirect evidence, such as that based on the mathematical modelling of cyclic AMP-Ca^{2+} interactions in pancreatic islets, suggests that cyclic AMP plays, at the most, a modest enhancing effect, in the normal process of glucose-induced insulin release[59].

b) Role of cyclic AMP in the hormonal stimulation of insulin release

Several hormones, e.g. pancreatic glucagon or enteric glucagon-like material[65], enhance glucose-induced insulin release. The physiological significance of such an effect is generally considered within the framework of an 'entero-insular axis' concept. According to this concept, the higher rate of insulin release evoked by oral as distinct from i.v. glucose loading is attributable, in part at least, to the release of gastro-intestinal hormones, which amplify the secretory response of the B-cell to circulating nutrients.

Several studies have documented that hormones, such as glucagon, indeed activate adenylate cyclase in islet homogenates[18, 33, 42, 71], increase the cyclic AMP content of intact islets[21, 61, 89] and enhance glucose-stimulated insulin release. The islet cells are also equipped with glucagon receptors[23, 69].

In other tissues, the hormonal activation of adenylate cyclase is mediated by the guanyl nucleotide regulatory protein Ns. A comparable situation was recently documented in pancreatic islets. Bernofsky and Amamoo first reported on the ADP-ribosylation of trichloroacetic-insoluble material in intact islets, but provided no

information on either the effect of microbial toxins upon ADP-ribosylation or the molecular weight of labelled peptides[2]. Svoboda et al.[85] demonstrated that cholera toxin catalyzes in islet membrane the ADP-ribosylation of two peptides with Mr 42,000 and 48,000 kDa, respectively, corresponding to the light and heavy forms of the α-subunit of Ns. In mirror image of the situation found in the exocrine pancreas, the toxin-stimulated ADP-ribosylation of the heavy form predominated in the islet membranes over that of the light form. In this study, the cholera toxin also stimulated the ADP-ribosylation of an unidentified 22-KDa peptide. The exposure of intact islets to cholera toxin is associated with typical changes in adenylate cyclase, including increased basal activity, augmented responsiveness to GTP (as distinct from stable GTP analogs, such as GTPγS) and decreased response to NaF[85]. Cholera toxin dramatically enhances the production and content of cyclic AMP in intact islets[24, 29, 85]. This effect is seen at both low or high glucose concentration, but an enhancement of insulin release is only observed at stimulating concentrations of the sugar[24, 29, 85, 95].

c) Role of cyclic AMP in the hormonal inhibition of insulin release

Adrenergic agents inhibit insulin release. The physiological significance of this effect is well documented. For instance, in stress or exercise, the rate of insulin release is indeed decreased and this could favour the mobilization of nutrients from extrapancreatic tissues[96]. Studies based on the use of selected adrenergic antagonists indicate that α_2-adrenergic receptors mediate the inhibitory action of catecholamines upon insulin release[43, 54, 66]. The presence of such receptors on islet cells was indeed documented[13, 14].

The inhibitory action of adrenergic agents on insulin release is suppressed when the islets are exposed in vivo or in vitro to the islet-activating protein isolated from the culture medium of *Bordetella pertussis*[37, 38]. This treatment results in typical changes in the activity of adenylate cyclase in islet homogenates or subcellular fractions[39, 58]. In addition to a decrease in basal enzyme activity, these changes include an increased responsiveness to GTP relative to stable GTP analogs, such as GTPγS, and resistance to the inhibitory action of α_2-agonists. It is indeed known that adrenergic agents inhibit islet adenylate cyclase[21, 33, 39, 42]. This effect is best seen in crude homogenates and in the presence of NaCl. It coincides with a decreased production of cyclic AMP in intact islets[21, 38, 62] and suggests the presence in islet cell of the regulatory protein Ni. This was recently confirmed by the demonstration that the islet-activating protein catalyzes in islet cell membrane the ADP-ribosylation of a single protein with a Mr close to 41 kDa, which corresponds to the α-subunit of Ni[58].

Incidentally, the significance of experimental results may be seriously obscured when epinephrine, instead of clonidine, is used to assess the effect of adrenergic agents upon adenylate cyclase activity in islet cells. For instance, Ullrich and Wollheim[90] recently reported that over a short-term incubation (3 min), epinephrine (used at an 0.1 µM concentration) increases the cyclic AMP content of islets exposed to forskolin (30 µM). However, in glucose-stimulated islets exposed to forskolin (30 µM), no increase in cyclic AMP content was evoked by the more specific α_2-agonist clonidine (0.1 µM). Moreover, the enhancing effect of epinephrine upon the cyclic AMP content of the glucose-stimulated islets exposed to forskolin was abolished by the β-blocker propranolol. Thus, when insulin release is adequately stimulated by D-glucose, the unexpected effect of epinephrine to augment the cyclic AMP content of islets exposed to forskolin apparently represents, to a large extent, a β-adrenergic effect and, as such, does not detract from the concept that α_2-agonists inhibit adenylate cyclase in islet homogenates and, under suitable experimental conditions, lower cyclic AMP production in intact islets. It should be underlined, however, that none of these data implies that the inhibition of insulin release by α_2-adrenergic agonists is attributable solely to inhibition of adenylate cyclase. Indeed, several authors have recently emphasized that such agonists also abolish glucose-induced insulin release by islets exposed to exogenous dibutyryl-cyclic AMP or 8-bromo cyclic AMP[40, 67, 90], as first reported 14 years ago[48]. These observations suggest that adrenergic agents may act at a step distal to the generation of cyclic AMP. For instance, it is conceivable that adrenergic agents favor the sequestration of Ca^{2+} in cellular organelles, independently, in part at least, of but in concert with their inhibitory action on adenylate cyclase[5]. The latter view is supported by the recent observations that, in intact islets exposed to ^{45}Ca, clonidine (10^{-9} M) favors the accumulation of the isotope in mitochondria and microsomes[1]. Incidentally, it had been proposed that adrenaline and noradrenaline increase K^+ permeability in islet cells[74], but this was not confirmed by either radioisotopic[86] or bioelectrical[15] criteria.

5. Conclusion

The present review emphasizes the concept that a rise in the cyclic AMP content of islet cells usually fails to cause any marked or sustained stimulation of insulin release in the absence of a suitable insulin secretagogue, but markedly enhances insulin release in stimulated B-cells. In the normal process of glucose-induced insulin release, a modest increase in cyclic AMP synthesis, possibly attributable to the activation of adenylate cyclase by Ca-calmodulin may play a limited enhancing role in the secretory response. However, changes in adenylate cyclase activity provoked by certain hormones may play an essential role in the rapid modulation of nutrient-stimulated insulin release by these environmental endocrine factors. In this respect, the B-cell appears to be equipped with specific receptor-transducer systems for the informational transfer of the input hormonal signal to the output catalytic response, including the guanyl nucleotide regulatory proteins Ns and Ni recently identified in islet cells. Cyclic AMP probably acts upon insulin release by activation of appropriate protein kinase(s) which may influence several variables of islet function, such as the intracellular distribution of Ca^{2+} and/or the sensitivity to cytosolic Ca^{2+} of Ca^{2+}-responsive targets.

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Protein phosphorylation in the pancreatic B-cell

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1. *Introduction*

The well-established modulatory role of cyclic AMP in insulin secretion (for review see Sharp[51]) affords presumptive evidence that protein phosphorylation mechanisms influence the release process. In view of the dependence of insulin secretion on Ca^{2+} and the growing awareness of the importance of Ca^{2+}-dependent protein kinases in regulating cellular activities, it was hypothesized[2] that protein phosphorylation might be the common mode of action of both Ca^{2+} and cyclic AMP in controlling insulin release. Demonstration of the presence of calmodulin in islets[18,56] supported the hypothesis that Ca^{2+}-calmodulin-dependent protein kinases are regulatory enzymes in the exocytotic release process. The discovery of Ca^{2+}- and phospholipid-dependent protein kinase and elucidation of the role of diacylglycerol in the regulation of its activity (for review see Nishizuka[38,39]) suggested that the enhanced inositol phospholipid breakdown seen in stimulated islets of Langerhans (for review see Best and Malaisse[4]) might also be linked to protein phosphorylation.

In this review we summarize the progress that has been made in characterizing islet protein kinases regulated by cyclic AMP, Ca^{2+} or diacylglycerol, and the as yet fragmentary data on the presence, localization and possible role of intracellular protein substrates for these kinases. We have therefore restricted ourselves to those enzymes which may be presumed to be involved in the control of insulin secretion. While it is possible that other cellular processes within the B-cell e.g. insulin biosynthesis are also regulated by phosphorylation–dephosphorylation, to date no studies have been performed in this area. Possible candidates for study in this context would be the casein kinases which have been demonstrated to be highly active in islets[55] but whose nature and mode of regulation are unknown.

In order to implicate protein phosphorylation as a regulatory mechanism in a cellular process such as secretion, the following criteria (adapted from Walsh and Cooper[60]) must be satisfied:

1. The presence of the particular protein kinase in the cells must be shown.
2. The cells must be demonstrated to contain endogenous substrate(s) for the kinase; and phosphatase(s) must be shown to be present to reverse the effect of the kinase.
3. In the intact cell it must be possible to demonstrate changes in extent of phosphorylation of such endogenous substrate(s) on stimulation of secretion.
4. The endogenous substrate(s) must be shown to bear a functional relationship to secretion; and changes in extent of phosphorylation must modify the activity of the protein substrate(s) in the secretory process.

For insulin secretion from the pancreatic B-cell, considerable progress has been made in demonstrating and characterizing various protein kinases in islets or B-cells, and in confirming the presence of endogenous protein substrates; protein phosphatase activity has been little studied, however. Thus the first two criteria have been at least partially met. As far as the implication of specific phosphoproteins in secretion is concerned, it has been possible in a few studies to demonstrate changes in phosphorylation occurring in conjunction with insulin secretion, but the nature of such proteins and their role (if any) in secretion has not been elucidated. Fulfilment of criteria 3 and 4 remains an elusive goal. The major protein kinase activities that have been studied in islets of Langerhans are listed in table 1.

2. *Methodological considerations*

We shall not present here detailed protocols for study of protein phosphorylation in subcellular fractions or intact insulin-secreting tissues; these can be found in the