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For debates

Cyclic ADP-ribose and the pancreatic beta cell: where do we stand?

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A model for the beta-cell stimulus-secretion coupling, postulating a central role for cyclic adenosine 5′ diphosphate ribose (cADPR) in glucose stimulated increase in cytoplasmic, free Ca^{2+} concentration ($[Ca^{2+}]_i$) has been proposed by Dr. Okamoto and colleagues. The origin of this model dates back to 1993 when the first report [1] and accompanying editorial in *Science* [2] depicted it as a significant advancement in our understanding of Ca^{2+}-signalling in these cells. In this model, cADPR releases Ca^{2+} from endoplasmic reticulum (ER) by activating the ryanodine receptor (Ry) and Ca^{2+} released from ER plays an important role in stimulating insulin secretion. Furthermore Dr. Okamoto maintains that beta cells do not possess a mechanism for Ca^{2+} release by inositol(1,4,5)trisphosphate ($Ins(1,4,5)P_3$) [1]. Through a series of studies the authors have produced evidence for a messenger role of cADPR and importance of Ry in glucose-stimulated increase in $[Ca^{2+}]_i$ [3]. Okamoto et al's interest in the NAD metabolite cADPR appears to stem from their earlier works where they demonstrate a crucial role for the cellular level of NAD in mediating diverse processes such as beta cell damage and beta cell tumour formation [4].

Background

Lee et al. [5] identified the structure of an active metabolite, i.e. cADPR responsible for the Ca^{2+}-releasing effect of β-NAD^+ in sea urchin eggs. It has since been demonstrated that cADPR also releases Ca^{2+} from intracellular non-mitochondrial stores in many mammalian cells [6]. These studies suggest that cADPR activates an intracellular Ca^{2+} channel having the pharmacological properties of Ry [7]. Early reactions to the discovery of cADPR was that this molecule is the endogenous ligand for Ry just as $Ins(1,4,5)P_3$ is the natural ligand for its receptor [8]. The enzymatic mechanisms for synthesis and degradation of cADPR have been characterized and the enzymes are known to be present in many cells. Despite this remarkable progress, some uncomfortable controversies have emerged regarding the physiological role of cADPR and even its Ca^{2+}-mobilizing effect. From this point of view, the case of the pancreatic beta cell is noteworthy because it was the first cell type where a physiological role for cADPR in mediating stimulus-secretion coupling was claimed [1]. In this commentary, we shall analyse the controversial aspects of cADPR in beta cells and attempt to identify possible reasons for such differences.

Does cADPR release Ca^{2+} from intracellular stores in insulin-secreting cells?

The claim that cADPR and not $Ins(1,4,5)P_3$ releases Ca^{2+} from intracellular stores in beta cells [1] was immediately questioned [9]. This controversy promoted other studies. To date, three groups have examined the role of cADPR in a range of insulin-secreting cells, e.g. RINm5F cells, *ob/ob* mouse beta cells [9], the glucose-responsive cell line INS-1 and rat islet cells [10–12]. The methods used in these studies for detecting Ca^{2+}-release by cADPR include use of permeabilized cells and measurements of $[Ca^{2+}]$ by Ca^{2+}-selective electrodes, or fluo-3 [9, 10], measurements of Ca^{2+}-activated K^+ current by patch-clamp

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Abbreviations. $[Ca^{2+}]$, Ambient free Ca^{2+} concentration; $[Ca^{2+}]_i$, Intracellular free calcium concentration; cADPR, cyclic adenosine 5′ diphosphate ribose; $Ins(1,4,5)P_3$, inositol(1,4,5)trisphosphate; Ry, ryanodine receptor; ER, endoplasmic reticulum; CICR, Ca^{2+}-induced Ca^{2+} release.

technique [9] and use of the microinjection technique [12]. Despite use of a variety of insulin-secreting cells, an array of sensitive methods, and different experimental approaches, neither a Ca^{2+}-releasing effect nor any other role of cADPR has been revealed in these studies.

Thus, to date, demonstration of Ca^{2+}-release by cADPR in insulin-secreting cells is limited to the studies carried out by Okamoto et al. In their experiments Ca^{2+} release by cADPR is highly reproducible. The reasons for such differences between results reported by Okamoto et al. and those reported by others have remained unclear. It is however obvious that the conditions of experiments where cADPR is reported to release Ca^{2+} are markedly different from those of experiments where it does not. Thus, Takasawa et al. [1] obtain Ca^{2+} release by cADPR when they use islet microsomes and an incubation buffer of high salt concentration. It is possible that in beta cells Ca^{2+} release by cADPR is demonstrable only from islet microsomes and only under particular incubation conditions. Studies that question Ca^{2+}-release by cADPR have not attempted to exactly duplicate the study of Takasawa et al. [1]. There seems, however, little incentive to do so. Such studies would require large numbers of islets which cannot be readily obtained. Moreover, the methods used in collection of large numbers of rat islets and preparation of microsomes from them, make it inevitable that significant contamination from alpha and delta cells, endothelial cells, fibroblasts, acinar cells and even lymph nodes, vessels and ducts may occur [13]. These latter cells may contain Ry in abundance (compared to low level of the receptor in beta cells) which may complicate interpretation of results obtained from islet microsome studies.

Other experimental conditions could also affect Ca^{2+} release by cADPR in different cells. Thus, Ca^{2+} release by cADPR characteristically requires calmodulin which may be diluted under conditions of experiments that do not obtain a release by the cyclic nucleotide [14]. Because of the uncertainty as to whether such critical conditions for cADPR action were perturbed in experiments with permeabilized cells and patch-clamp experiments, Webb et al. [12] directly examined the effect of cADPR under more physiological conditions using intact beta cells and the microinjection technique. It was still not possible to detect a rise in $[Ca^{2+}]_i$ by microinjected cADPR. Furthermore, microinjection of a cADPR-antagonist did not block a glucose-induced rise in $[Ca^{2+}]_i$ [12]. Essentially similar results have been reported by Willmott et al. [11] in rat beta cells.

Rutter et al. [10] reported a small increase in ambient $[Ca^{2+}]$ after addition of cADPR and this they attributed entirely to contamination of cADPR with Ca^{2+}. Investigators working with other cell types have also been concerned with the possibility of contamination of cADPR with Ca^{2+} and this prompted them to conduct a series of control experiments to rule this out [15].

Ins(1,4,5)P_3 as Ca^{2+}-mobilizing second messenger in beta cells

According to the studies of Takasawa et al. [1], Ins(1,4,5)P_3 is not a calcium-mobilizing second messenger in beta cells. This view is clearly at odds with the standard view. According to the authors, early studies demonstrated Ca^{2+} release by Ins(1,4,5)P_3 from microsomes or permeabilized cells because those studies used transformed cells. Some support for this view is available from immunocytochemical studies of beta cells [16] and experiments that directly compared the Ins(1,4,5)P_3-induced Ca^{2+} release in tumoural and native beta cells [10]. The latter experiments confirm that Ins(1,4,5)P_3 releases much less Ca^{2+} from native beta cells as compared to the tumoural ones [10]. In spite of these, the notion that beta cells entirely lack mechanisms for Ca^{2+} release by Ins(1,4,5)P_3, is inconsistent with numerous studies demonstrating formation of Ins(1,4,5)P_3 on stimulation of phosphoinositide-specific phospholipase C-linked receptors and release of Ca^{2+} from intracellular stores by the inositol trisphosphate in permeabilized cells [17–20].

Ryanodine receptor in insulin-secreting cells

The mechanism of Ca^{2+} release by cADPR in any cell involves an indirect effect of the cyclic nucleotide on Ry. However, convincing evidence to support the view that Rys play significant role in Ca^{2+}-signalling in beta cells, is still lacking. In intact or permeabilized insulin-secreting cells, caffeine, an agonist of Ry, releases little or no Ca^{2+} from intracellular stores [9, 10, 17]. Activating concentrations of ryanodine or its β-alanyl analogue also do not yield a detectable increase in $[Ca^{2+}]_i$ in beta cells [21, 22]. Gromada et al. [23] demonstrated evidence for involvement of Ry in glucagon-like peptide-I-induced increase in $[Ca^{2+}]_i$ in insulin-secreting cells, while others have presented conflicting evidence [24]. Nevertheless, there are bits and pieces of data suggesting the presence of a Ry-like channel in insulin-secreting cells, albeit at a low level [18, 22, 25]. Islet from *ob/ob* mice, insulin-secreting β-TC3 cells and RINm5F cells express mRNA for Ry [26, 27]. Moreover, this Ry appears to be type 2, a type that according to some reports, is sensitive to cADPR [27, 28]. It is possible that low and perhaps labile expression of Ry in beta cells together with subtle differences in the experimental conditions used by different investigators accounts for different conclusions drawn regarding the existence of Rys and their importance in beta cells.

cADPR-binding protein/receptor

A recent claim that FKBP12.6 may be the intracellular receptor for cADPR is a novel one [29]. However, we need to see whether this finding can be reproduced. It may be noted that earlier studies that identified cADPR-binding proteins by photoaffinity labelling did not detect any protein with a molecular weight of FKBP12.6 [30].

cADPR level in glucose-stimulated beta cells

Does glucose stimulation increase cADPR level in beta cells? According to Takasawa et al. [1], it does. The authors prepared extracts from beta cells treated in high glucose conditions and demonstrated that these extracts released more Ca^{2+} from islet microsomes compared with that released by extracts prepared from low glucose-treated cells. Although this was the only way to address the question at a time when no other suitable method was available for cADPR measurement, we do not believe that any meaningful conclusion can be drawn from such experiments. This is because extracts prepared from high glucose-treated cells, are likely to contain not only high levels of Ca^{2+} to start with but also other Ca^{2+}-mobilizing agents such as $Ins(1,4,5)P_3$ and arachidonic acid. Recent studies that have measured cADPR in beta cells by radioimmunoassay have not been able to demonstrate a detectable rise in cADPR level on stimulation by glucose [31].

Role of intracellular Ca^{2+} stores in glucose-stimulated insulin secretion

Ca^{2+} entry through voltage-gated Ca^{2+} channels appears to be essential for increasing $[Ca^{2+}]_i$ after stimulation by glucose [19]. Against this background, the role of glucose in releasing Ca^{2+} from intracellular stores and thereby stimulating secretion appears quantitatively less important. Thus, although Ca^{2+}-mobilizing signalling molecules such as $Ins(1,4,5)P_3$ and arachidonic acid are generated on stimulation by glucose [20, 32], it has seldom been possible to demonstrate directly that glucose stimulation causes net release of Ca^{2+} from intracellular stores. Numerous studies have demonstrated that when $[Ca^{2+}]_i$ is raised solely by release of the ion from intracellular stores, there is only a small and transient stimulation of insulin secretion [33, 34]. Furthermore, depletion of intracellular Ca^{2+} stores by thapsigargin appears to have little effect on glucose-stimulated Ca^{2+}-oscillation or insulin secretion [35]. This does not rule out the possibility that Ca^{2+} entering through the voltage-gated Ca^{2+} channels is amplified by Ca^{2+}-induced Ca^{2+} release (CICR) [18], a process that could be modulated by $Ins(1,4,5)P_3$ or cADPR. To what extent such amplification occurs and the quantitative importance of different Ca^{2+} release channels in mediating CICR remain to be carefully studied.

cADPR-metabolizing enzymes in beta cells

Beta cells express two bifunctional enzymes CD38 and bone marrow stromal antigen 1 (BST-1) involved in synthesis and degradation of cADPR [36]. CD38 catalyses at its extracellular domain the synthesis of cADPR from β-NAD^+ as well as hydrolysis of cADPR to ADP ribose. Transgenic mice, overexpressing CD38 in beta cells manifest increased insulin secretion in response to glucose [3]. This has been attributed to increased formation of cADPR by CD38 and consequent increased release of Ca^{2+} from intracellular stores. CD38, however, appears to be involved in many physiological functions and other mechanisms by which it might affect secretion may need to be considered. For instance, CD38 can possibly act as an ADP-ribosylating enzyme and may induce insulin secretion by ADP-ribosylation of proteins including G-proteins [37, 38]. Whether in transgenic mice, CD38 overexpression may enhance insulin secretion by subtle effects on development and growth of beta cells also needs to be explored.

Difficulties in demonstrating Ca^{2+}-release by cADPR in other cells

The controversy regarding the Ca^{2+}-mobilizing effect of cADPR and its significance is not unique to beta cells. In the heart, it has been demonstrated that cADPR releases Ca^{2+} from isolated sarcoplasmic reticulum vesicles and activates Ry incorporated in the planar lipid bilayer [28]. However, the results could not be reproduced by others [39, 40]. Liver cells do not seem to express mRNA of any of the known Rys, but cADPR has still been reported to release Ca^{2+} from nuclear envelops of these cells [41]. On the other hand Lilly and Gollan [42] demonstrate that cADPR does not release Ca^{2+} from hepatic microsomes. The list could be longer.

Conclusions

Dr. Okamoto's hypothesis that cADPR is a signalling molecule coupling glucose metabolism to increase in $[Ca^{2+}]_i$ in beta cells is interesting. The doubts derive from a failure by other investigators to reproduce their main finding, i.e. Ca^{2+}-release by cADPR in beta cells. Even if it is accepted that cADPR might release Ca^{2+} from intracellular stores in beta cells, the stand taken by Dr. Okamoto seems exaggerated

when he regards this mechanism as one of primary importance. However, the intriguing observations made in studies with transgenic mice, may point to some modulatory role of cADPR in insulin secretion. Whether cADPR and CD38 may affect secretion in beta cells by mechanisms unrelated to the postulated Ca^{2+}-mobilizing effect needs to be explored. At present Ca^{2+} release by cADPR and its role as a messenger molecule, remain controversial even in cells where Rys are expressed in plentiful numbers. Such controversies should provide incentives for future studies directed at molecular characterization of intracellular Ca^{2+} channels and elucidation of their quantitative importance in mediating CICR in beta cells.

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