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The enzyme glucosidase II is induced in response to amino acid deprivation in renal epithelial cells

In a recent paper in the Biochemical Journal [1], we described the identification and partial characterization of a 110 kDa protein in bovine renal epithelial cells that shows a major induction in response to amino acid deprivation. We were unable to assign a function to this protein, and routine database searches failed to identify it with any known sequence. Recent information now strongly suggests that this protein is in fact the endoplasmic-reticulum enzyme glucosidase II.

Glucosidase II was isolated from rat liver in 1996 [2] and was shown to contain a glycosylated enzymically active α-subunit of about 110 kDa and a β-subunit of 58 kDa, which contains an HDEL endoplasmic-reticulum retention sequence. Partial sequence data derived from the purified enzyme identified it with a human clone of unknown function (accession no. D42041). Glucosidase II has now been cloned and sequenced from mouse T-cells as a protein which associates with CD45 [3] (accession no. U92793), and a full cDNA sequence for human glucosidase has recently been deposited in the database (accession no. AJ000332). The amino acid sequences of the α-subunits in each case are closely similar. The protein from human and mouse sources have a 32-amino-acid signal sequence followed by the sequence VDRSNFKT (amino acids 33–40), which represents the N-terminus of the mature protein. This is closely similar to the N-terminus of the protein which we found to be induced in renal epithelial cells (VDRINFKT..). The molecular mass of the bovine renal protein, its localization in the post-mitochondrial pellet, its high affinity for DEAE-cellulose and hydroxyapatite and its purification on concanavalin A–Sepharose in our investigation are very similar to the properties reported for rat liver glucosidase II in [2]. The internal sequence which we derived (SPLIELNFPLS) has similarity to amino acids 411–421 in the mouse sequence (PPLFSLGYHQS). Owing to the major problems which we encountered in purifying the protein, it is likely that our internal sequence may not be completely correct. It is thus very probable that the novel protein which we identified in [1] is the α-subunit of glucosidase II.

According to [4], glucosidase II has an important function in the folding and maturation of glycoproteins. First, acting after glucosidase I, it removes the second two glucose residues from the initiating polysaccharide and thus allows the nascent protein to bind to the proteins calnexin or calreticulin, which act as lectins and retain the nascent glycoprotein in the endoplasmic reticulum while folding and oligomeric assembly occur. Secondly, it removes the innermost glucose residues and thus releases glycoprotein from calreticulin or calnexin. In this context it is of particular interest that expression of calreticulin has also been shown to be greatly induced by amino acid deprivation in renal epithelial cells [5].

The rationale of the induction of calreticulin and glucosidase II in response to amino acid deprivation is so far unclear. It is tempting to speculate that the induction of these proteins promotes an increased synthesis and membrane insertion of glycoproteins such as amino acid transporters or activators of these transporters and that this tends to maintain amino acid concentrations in cells when the extracellular amino acid supply is reduced.

John. D. McGIVAN

Department of Biochemistry, School of Medical Sciences, University Walk, Bristol BS8 1TD, U.K.

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Of mice and men (and rats): caveats in defining specific roles of GTP in the pancreatic islet

In their recent publication [1], Detimary and colleagues re-examine the use of mycophenolic acid (MPA) as a probe to selectively deplete the GTP content of insulin-secretory cells. They were unable to dissociate GTP from concomitant changes in ATP (or ATP/ADP ratio), and concluded that 'MPA is not an adequate tool for evaluating a specific role for guanine nucleotides in the control of insulin secretion.' On the basis of two shortcomings in their publication, we believe their conclusion to be erroneous: (1) their citation of extant studies using MPA is selective, and is striking in its omission of many relevant observations which do not support their conclusion; and (2) mouse islets appear to be inappropriate as a model system to test the hypothesis and therefore they are 'comparing apples with oranges' when relating their findings to previous publications.

We would like to cite the following cogent studies [2–8] using MPA, which support a selective role of GTP in the physiological control of insulin release.

In none of the four other β-cell preparations studied previously does MPA decrease the ATP/ADP ratio as it does in mouse islets; in fact, it frequently elevates this parameter slightly. Nonetheless, it potently inhibits insulin release in each of these preparations. Furthermore, the effects of MPA on insulin release are closely mimicked by mizoribine, which likewise depletes GTP but fails to decrease the ATP/ADP ratio.

MPA (or mizoribine) does modestly decrease the ATP content in insulin-secreting preparations. However, provision of adenine or adenosine to all preparations with the sole exception of mouse islets, reverses the effect on ATP yet fails to restore insulin release. Furthermore, the provision to rat islets of either L-alanosine or 6-methylmercaptopurine (which selectively inhibits ATP synthesis to an extent similar to MPA but without reducing GTP) has no effect on insulin secretion [3]. Similar effects are seen with glutamine antagonism [3].

Table 1   Effects of an overnight culture period in the absence or presence of MPA (25 μg/ml) on the content of purine nucleotides in intact human islets

Human islets were generously supplied by Dr. D. Scharp and T. Mohanakumar of the Islet Isolation Core, Washington University, St. Louis, MO, U.S.A. They were incubated overnight (as in [2]) in the presence of 25 μg/ml MPA or diluent and analysed for purine nucleotides using HPLC [2]. Data are from four independent experiments involving a total of five to eight determinations per nucleotide. Abbreviation: ns, not statistically significant.

	Control		MPA
[GTP] (pmol/islet)	2.63 ± 0.39	$\leftarrow -73\% \rightarrow$ ($P = 0.002$)	0.72 ± 0.21
GTP/GDP ratio	5.56 ± 0.80	$\leftarrow -74\% \rightarrow$ ($P = 0.02$)	1.45 ± 0.50
[ATP] (pmol/islet)	11.63 ± 1.6	$\leftarrow -22\% \rightarrow$ ($P = $ ns)	9.05 ± 1.65
ATP/ADP ratio	6.35 ± 2.37	$\leftarrow -10\% \rightarrow$ ($P = $ ns)	5.69 ± 1.94

Additionally, MPA inhibits insulin release induced by protein kinase C activation [2], yet it is unlikely that this pathway would be sensitive to the modest declines in ATP or ATP/ADP ratio induced by MPA in mouse islets. More cogently, MPA can inhibit glucose-induced insulin secretion even when ATP-sensitive K^+ channels (the major β-cell effector of changes in the ATP/ADP ratio) are kept open using diazoxide [4]; this effect correlated well with changes in GTP/GDP but not at all with ATP/ADP ratios, which did not change.

Clearly, the effects of MPA (or of mechanistically related drugs) are not attributable to changes in adenine nucleotides, as postulated by Detimary et al. [1]. Indeed, in a large number of studies in other non-endocrine cell types, MPA or mizoribine generally had only minimal effects on adenine nucleotides (see the Discussion in [2]). Additionally, it should be noted that some, if not all, of the cellular effects of MPA in β-cells can be explained by inhibition of the activation of specific GTP-binding proteins [7,7a]. This finding links changes in GTP (but not ATP) induced by MPA to the effects observed on signal transduction (such as inhibition of phospholipase C; [5]) in rat islets.

Secondly, it is important to note that these authors [1] studied female mouse islets, whereas our group reported [2–8] studies from intact male rat islets, human islets (Table 1) and two transformed β-cell lines (INS-1 cells and HIT-TI5 cells). Our findings in these four models are internally consistent and concordantly indicate that MPA can selectively reduce GTP/GDP without reducing ATP/ADP; the consonance between these models crosses species and cell preparations and clearly identifies mouse islets as the 'outlier'. It is therefore relevant that mouse islets have been reported to have a number of striking anomalies in signal transduction and in insulin release compared with human or rat islets (cf. [9,10]); these may extend to NMRI mice [10], used in the study by Detimary et al. [1]. As Grodsky and colleagues pointed out [10], 'these differences are consistent even when a variety of mouse and rat strains are compared. Thus, it is quite possible to extrapolate data inappropriately from one species to another when defining mechanisms of regulation of insulin secretion.'

However, most striking in the current studies by Detimary's group [1] is the very weak effect of MPA on the GTP content of mouse islets (-29 to 41%) compared with all other β-cell preparations (-73 to -81%) and many other cell types studied. Furthermore, the failure of adenine or adenosine to restore adenine nucleotides in mouse islets (in stark contrast with rat islets, isolated β-cells and other cell types studied) strongly suggests a defect in the transport and/or salvage of nucleobases and nucleosides in the murine islet; we are therefore not surprised that mouse islets demonstrate anomalies of adenine nucleotide metabolism in response to MPA! More importantly, this defect in adenine salvage precluded Detimary's group from examining critically the role they postulated for changes in ATP or ATP/ADP in mediating the effects of MPA. Thus, since the authors were unable to dissociate ATP or ATP/ADP ratio from GTP (GTP/GDP values were not measured), their hypothesis simply could not be tested in their murine islet model.

It should also be noted that Detimary's group assessed nucleotide content of islets only after 60 min of priming plus 65 min of incubation (both at high glucose concentrations) in bicarbonate buffer. Such prolonged incubations profoundly alter purine nucleotides in islets [11,12] and might well have perturbed the physiological temporal relationships between nucleotides and the acute induction of physiological insulin release. Furthermore, concern might be expressed about the specificity of the multi-step enzymic assays for nucleotides used by Detimary's group when analysing complex biological samples (although it apparently is accurate and specific when using standard solutions of nucleotides). The high concentrations of hexokinase and long incubation times used to remove ATP from the samples would likely permit utilization of some of the UTP as well [13]. Thus there is the possibility that the accumulating UDP might inhibit nucleoside diphosphokinase [14], which is used in the next step to transform GTP into ATP for subsequent quantification; UDP in concentrations as low as 1–2.5 mM inhibits that enzyme by $\geqslant 40$–50% [14]. Similarly, the pyruvate kinase added to convert ADP into ATP in the first step might spuriously increase GTP [15] in the subsequent steps. These uncertainties assume a central relevance when one considers that MPA not only profoundly inhibits GTP levels but also increases UTP content just as dramatically [2].

Given all the above findings, we maintain that the studies cited are inadequate to dismiss the utility of agents such as MPA to decipher the normal physiological cellular role(s) of GTP (as seen in rat and human islets and isolated β-cells). In fact, given the fact that a selective inhibition of ATP synthesis (using mitochondrial poisons such as antimycin) secondarily decreases GTP/GDP and GTP content profoundly [14], we speculate that the close parallelism of GTP levels with those of ATP in β-cells (which we also noted several years ago; [14]) may reflect the role of GTP as a distal effector of some of the actions of ATP, rather than the converse (as postulated by Detimary et al. [1]).

Note added in proof (received 26 September 1997)

Recently, another group of investigators has also reported [16] that MPA inhibits glucose-induced potentiation of insulin release from isolated rat islets, even under conditions of Ca^{2+} deprivation. This inhibitory effect was dependent on the depletion of guanine nucleotides, but occurred in the absence of changes in adenine nucleotides, findings which support our formulation.

Stewart A. METZ*†[1] and Melissa MEREDITH*

*University of Wisconsin Hospital and Clinics H4/568 CSC, 600 Highland Avenue, Madison, WI 53792-5148, U.S.A., and †The Middleton Veterans Administration Hospital, Madison, WI 53705, U.S.A.

[1] To whom correspondence should be addressed.

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Much ado about mycophenolic acid

We thank Dr. Metz and Dr. Meredith for their interest in our recent work with mycophenolic acid (MPA) [1]. Some of their comments [2], however, call for a reply to avoid fostering of any misunderstanding.

Metz and Meredith [2] cite seven of their own publications using MPA [3–9] to support a selective role of GTP in the physiological control of insulin release. The first three [3–5] are the most relevant and were cited in our paper [1]; another one is a proceedings report [9], one was still in press [7] and the last two [6,8] do not provide additional arguments in support of the selectivity of action of MPA.

The conclusion that a biological process is regulated by changes in the GTP concentration often rests on the observations that MPA impairs this process while lowering GTP levels, and that both effects are reversed by guanine and not by adenine. In fact, this confirms that MPA decreases guanine nucleotide synthesis but does not prove that the fall in GTP is the direct mediator of the observed biological effect. Thus inhibition of IMP dehydrogenase (by MPA or other drugs) perturbs the equilibrium between nearly all nucleotides [10]. UTP levels increase, and this increase is corrected by guanine [3]. The marked increase in IMP levels can also be reversed by guanine, in spite of persistent blockade of IMP dehydrogenase, because the restoration of GTP levels permits IMP transformation into AMP by adenylosuccinate synthetase, for which GTP serves as cofactor [11]. Adenine nucleotides may also be affected to various extents [10,12]. In the experiments performed by Metz's group, MPA treatment of the islets caused a 35–45 % decrease in total ATP [3–5]. The adjective 'modest' that is repeatedly used to qualify this decrease seems inappropriate to us. Moreover, the decrease in insulin content [20–30 %] that MPA also caused in rat islets [3,13] seriously complicates the comparison of total nucleotide levels or ratios in these treated islets with those measured in control islets. Thus insulin granules contain substantial amounts of nucleotides with a low ATP/ADP ratio close to 1 [14]. A decrease in the cytoplasmic ATP/ADP ratio may thus pass unnoticed when the granular pool of nucleotides has been decreased [14]. This pitfall did not exist in our mouse islets in which MPA did not influence the insulin content [1].

The suggestion [2] that mouse islets might be poorly permeable to nucleosides and nucleobases is not correct. This has been shown directly for adenosine [15]. Moreover, in a novel series of experiments in which adenine was present during the culture with MPA and then for two successive incubations of 1 h without MPA, ATP levels were restored (Table 1). However, the decrease in the ATP/ADP ratio was not corrected, and this might explain why insulin release was still inhibited (results not shown).

Metz and Meredith [2] also express several concerns about our methodological approach [2]. We attribute these critiques to an insufficiently detailed description of our assays and provide the following three clarifications. First, the timing of our incubations is justified by our previous report that the changes in nucleotide levels or ratios that glucose produces within 5 min remain stable for up to 60 min [16]; in addition, our approach permits measurements of insulin secretion and nucleotides in the same samples. Secondly, because of dilution of the sample extract, the transformation of GTP into ATP is performed in the presence of, at the most, 10 nM UDP, a concentration considerably less than those that might affect nucleoside diphosphate kinase [17]. Thirdly, no spurious increase of GTP by pyruvate kinase may invalidate our measurements because adenine and guanine nucleotides are measured in separate aliquots from the same extract; after the pyruvate kinase reaction, ATP is either measured or first destroyed before transformation of GTP into ATP.

The critical issue of this debate is whether changes in GTP or the GTP/GDP ratio occurring under physiological conditions exert a specific role in insulin secretion. We maintain that MPA is not an adequate agent to establish such a role. As shown in the very first study of Metz's group [3] and unambiguously restated subsequently [13], 'insulin release from rat islets treated with MPA is not inhibited until GTP content falls by more than 80 % …'. If we bear in mind that some of the residual GTP is contained within organelles, the cytoplasmic concentration must be extremely low. Does this ever occur in the absence of MPA? To the best of our knowledge, Metz and Meredith have reported acute effects of glucose on GTP and the GTP/GDP ratio in insulin-secreting cells only in the presence of high K^+ and diazoxide [5]. An increase in GTP from $\approx$ 2.0 to $\approx$ 2.8 pmol/islet was observed between 3.3 and 16.7 mM glucose. We found a similar 33 % difference between mouse islets incubated in a physiological medium (normal K^+) containing 3 and 20 mM glucose [16]. Such differences are far smaller than 80 % and should thus be insufficient to cause changes in insulin release according to Metz's group. Others of their data also indicate that there is no correlation between GTP and insulin secretion. Comparison of Figures 6 and 8 in [3] shows that supplementation of the culture medium with 50 μM guanine prevented the

Table 1   Effects of adenine on ATP and GTP levels in mouse islets treated with MPA

Islets were cultured for 18 h in RPMI medium containing 10 mM glucose with or without 25 μg/ml MPA before being incubated for two successive periods of 60 min in a medium containing 15 mM glucose without MPA. Adenine (250 μM) was present as indicated. Islet nucleotides were measured at the end of the second period of incubation. Values are means $\pm$ S.E.M. for 15 batches of islets from three separate experiments. *$P < 0.001$ for the difference from controls (first row).

Addition during:		Nucleotide content after incubation (pmol/islet)			
Culture	Incubation	GTP	ATP	ADP	ATP/ADP ratio
None	None	5.5 ± 0.2	19.1 ± 0.6	2.0 ± 0.1	10.2 ± 0.7
MPA	None	3.3 ± 0.1*	14.2 ± 0.4*	2.6 ± 0.1*	5.7 ± 0.3*
MPA + adenine	Adenine	3.8 ± 0.2*	18.8 ± 0.7	2.6 ± 0.1*	7.2 ± 0.3*

inhibition of glucose-induced insulin secretion, although GTP levels were restored to 70 % only (and ATP to 85 %).

In conclusion, we share the opinion of Metz and Meredith [2] that guanine nucleotides may play a specific role in the regulation of insulin secretion, but disagree that this role (or that of adenine nucleotides) has been definitively proved. Our own reports show that insulin secretion stimulated by increasing glucose concentrations similarly correlates with increases in the ATP/ADP and GTP/GDP ratios in the same islets [16,18]. However, we found MPA unsuitable as a means to assess the genuine effects of GTP and reported these negative results with the hope of boosting new approaches to study how both types of purine nucleotides are really involved in stimulus–secretion coupling in β-cells.

Philippe DETIMARY and Jean-Claude HENQUIN[1]

Unit of Endocrinology and Metabolism, University of Louvain Faculty of Medicine, UCL 55.30, Avenue Hippocrate 55, B 1200 Brussels, Belgium

[1] To whom correspondence should be addressed.

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