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Note added in proof. The three-dimensional structure of the HLA-DR1 molecule has recently been reported (Brown et al. (1993) *Nature* 364: 33–39) and shows many similarities to published MHC class I structures. The three principles of peptide binding outlined above seem to hold. Likewise, the ends of the two α -helices are closer to the β -sheet floor, permitting longer peptides to bind and allowing for the formation of a salt bridge between α 76Arg and β 57Asp, directly under the bound peptide.

Response from the author

Dear Sir,

In reply to Dr. Papadopoulos, I would agree with him that we can only make guesses about the manner in which class II HLA antigens and the peptides they bind interact. As he points out, the evidence favours a model in which class II antigens may bind much longer peptides than do class I antigens. The anchoring is presumed to be by aminoacid residues that hydrogen bond to various parts of the HLA class II structure. In our model we chose charged residues belonging to the third hypervariable region of DR or DQ- β molecules as likely discriminators which might either assist or repel binding of a peptide such as ABBOS by hydrogen bonding or by charge repulsion or attraction. The actual binding may or may not be mediated through these "discriminator" residues, but in our hypothesis they strongly influence the binding function.

Teleologically such discriminators must exist otherwise specificity and selection of peptide binding by class II alleles would not be

a reality. As Dr. Papadopoulos remarks, "the alignment of the ABBOS peptide in the antigen binding groove must be performed again" when we know more about anchor specificities of class II molecules.

We all look forward to the day when this is possible.

Yours sincerely,

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Evolution of the glucokinase glucose sensor paradigm for pancreatic beta cells

Dear Sir,

In a recent review [1] Dr. P.J. Randle makes the claim of having discovered glucokinase in the pancreatic islet [2] and relegates to the confirmatory category our report which demonstrated directly, and for the first time, the presence of this enzyme in pancreatic islet tissue [3]. Dr. Randle's account distorts historical facts. A correction is in order.

Studies of Grodsky and collaborators [4] and of Coore and Randle [2] were the first to surmise in the early 1960s, that islet beta cells might be freely permeable to hexoses including D-glucose and that sugars, which are suitable substrates for hexokinases and thus glycolysis, are capable of eliciting insulin release. They suggested a link between stimulant fuel catabolism and hormone releasing function in beta cells. However, the approach taken by these investigators was incapable of distinguishing which of the four known glucose phosphorylating enzymes might be involved, because their studies were indirect and because the inhibitors of hexokinases (D-mannoheptulose and 2-desoxyglucose) lacked specificity or were ineffective at the concentrations they applied. Attempts by Ashcroft and

Randle from 1968 [5, 6] to resolve this issue by direct analysis of glucose phosphorylation in homogenates of isolated islets, seemed to show the presence of low K_m hexokinase activity but a lack of glucokinase. Our first publication on this topic appeared in May 1968 [3], and demonstrated that islet tissue contained both (a) low K_m hexokinase(s) and a high K_m hexokinase (which we classified as glucokinase). This paper also reported the presence of glucose 6-phosphatase, confirming the data of Taljedal [7]. Additionally it provided the first clear direct evidence that intra- and extracellular glucose of islet cells equalized within minutes of an i. v. glucose load thus resembling liver cells. Glucose loading caused elevations of islet glucose 6-phosphate and fructose 1,6-bisphosphate presumably a manifestation of activation of glycolysis.

On the basis of these data we were able to formulate a plausible concept of beta cell glucose metabolism involving glucokinase as a key element [3]:

"The scheme of carbohydrate metabolism of the islets of Langerhans, emerging from these and other studies, has great similarities to the situation found in liver. The membrane properties of β -cells, the probable presence of two glucose phosphorylating enzymes with high and low affinity for the substrate, and the ability to liberate free glucose from glucose 6-phosphate constitute ideal conditions for rapid adjustments of glycolysis to blood glucose levels."

This publication and two related papers [8, 9] resulted from the systematic application of the powerful technology of quantitative histochemistry developed by O. H. Lowry and his associates and was made feasible by a novel oil well method and enzymatic cycling to determine enzymes and metabolites of glucose metabolism in microscopic structures [10] as, for instance, the glucose sensitive insulin secreting pancreatic islets.

Following our 1968 report Ashcroft and Randle evidently improved their methodology to measure up to the difficult task at hand and in 1970 [11] they confirmed the presence of glucokinase in islets correcting their earlier erroneous findings [5, 6].

We feel that it is not presumptuous to claim, that our laboratory deserves the credit for discovering glucokinase in islet tissue and that it has played a leading role in developing the glucokinase glucose sensor paradigm. It seems fitting to acknowledge here that during its early stages our research was strongly influenced by the work of Cahill [12], Weinhouse [13] and Sols [14] on glucose metabolism in liver, by the physiological studies of Grodsky with the isolated perfused rat pancreas [15], and by the methodological contributions of the Lowry School [10] which made measurements in microscopic structures possible. More recently the discoveries of molecular biologists [16, 17] and human geneticists [18], have added an exciting, entirely new dimension to this field of diabetes research. We have used several opportunities to review and discuss our contributions and those of others in the field [19–22].

Respectfully,
F.M. Matschinsky

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Response from the author

Dear Sir,

Thank you for the opportunity to respond to Dr. Matschinsky's letter in relation to my review article entitled "Glucokinase and candidate genes for Type 2 (non-insulin-dependent) diabetes mellitus" [1].

The review did not claim that Coore and Randle (1964) [2] discovered glucokinase (GK) in pancreatic islets. What it did claim, and correctly, was that Coore and I [2–4] were the first to suggest that GK is the glucoreceptor for glucose induced insulin release and to provide evidence for it. Matschinsky and Ellerman (1968) [5] were the first to provide direct evidence for intracellular glucose and GK in pancreatic islets as my article also makes clear. Shortage of space prevented me from mentioning that their studies were on islets of obese hyperglycaemic mice – not normal mice; and that the relevance of their findings to normal islets was not established, nor indeed alluded to, by them. Ashcroft and Randle (1969, 1970) [6, 7]

were therefore the first to address the more difficult and important problem viz. to show the presence of GK in normal mouse islets. This was difficult (not erroneous as suggested by Dr. Matschinsky) because the ratio of GK to hexokinase was less favourable. Success was eventually achieved by specifically inhibiting hexokinase with excess glucose 6-phosphate which also had the advantage of nullifying possible interference by glucose 6-phosphatase (our assay method followed the conversion of [1-¹⁴C] glucose to [1-¹⁴C] glucose 6-phosphate which was diluted out by the unlabelled glucose 6-phosphate). This was important. The discovery by Ashcroft and Randle (1968, 1970) [7, 8] that islet glucose 6-phosphatase is inhibited by glucose (competitive K_i 8.9 mmol/l [7]), meant that a mixture of soluble and particulate hexokinase plus particulate glucose 6-phosphatase could, in theory, simulate the kinetics given by a mixture of hexokinase and GK. It is possible therefore that the conclusions of Matschinsky and Ellerman were erroneous; their non-radioactive assay precluded inhibition of hexokinase with glucose 6-phosphate or dilution out of the effects of glucose 6-phosphatase. To the best of my

knowledge this point has not been referred to by Dr. Matschinsky and I have not published it before.

In 1971–1973 Dr. Matschinsky and his colleagues published a series of papers purporting to show that stimulation of insulin release by sugars is not dependent on their metabolism and therefore that GK could not be the glucoreceptor i.e. they disowned the GK paradigm which he claims to have discovered. The following quotations are illustrative: in the summary of Landgraf et al. (1971) [9] “it is postulated that a glucoreceptor of broad specificity is involved in the mechanism of insulin release and that metabolism of glucose is not an essential part of the releasing action of this sugar”; in the summary of Matschinsky et al. (1971) [10] “These results and reports by other investigators suggest that glucose itself rather than its metabolites stimulate insulin secretion”; and in Matschinsky and Ellerman (1973) [11] “our current working hypothesis is that glucose acts by stimulating glucoreceptor molecules located in the β -cell membrane”. Dr. Matschinsky’s eventual reconversion to the GK/glucoreceptor hypothesis was effected from 1973 onwards mainly as a result of others failing to confirm his evidence purporting to show that metabolism of sugars and their insulin secretory effects in islets can be dissociated. I have already summarised in the review Dr. Ashcroft’s later work on N-acetylglucosamine which in my opinion clinched the GK/ glucoreceptor hypothesis; I have acknowledged the importance of Dr. Matschinsky’s later work in the 1980s; and I have reviewed the important molecular biological studies including the most important discovery of GK mutations.

Those who reviewed my article before acceptance for publication were kind enough to say that it was balanced and certainly the intent was to give a brief and accurate historical statement. Dr. Matschinsky has yet to tell us why he consistently failed to cite the paper of Coore and Randle (1964) until a publication in 1971 [10] in which he switched his allegiance to the direct glucose receptor hypothesis. The work which Dr. Coore did as a graduate student was pioneering in so many ways; and in addition to the preparation which he developed, and the discoveries mentioned here and in the review, he also discovered the inhibition of insulin secretion by adrenaline [2, 4]. Dr. Coore is currently Professor of Biochemistry in the University of the West Indies in Trinidad.

Yours sincerely,
P.J. Randle

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