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Microcompartmentation of Energy Metabolism at the Outer Mitochondrial Membrane: Role in Diabetes Mellitus and Other Diseases

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Complexes made up of the kinases, hexokinase and glycerol kinase, together with the outer mitochondrial membrane voltage-dependent anion channel (VDAC) protein, porin, and the inner mitochondrial membrane protein, the adenine nucleotide translocator, are involved in tumorigenesis, diabetes mellitus, and central nervous system function. Identification of these two mitochondrial membrane proteins, along with an 18 kD protein, as components of the peripheral benzodiazepine receptor, provides independent confirmation of the interaction of porin and the adenine nucleotide translocator to form functional contact sites between the inner and outer mitochondrial membranes. We suggest that these are dynamic structures, with channel conductances altered by the presence of ATP, and that ligand-mediated conformational changes in the porin–adenine nucleotide translocator complexes may be a general mechanism in signal transduction.

KEY WORDS: Benzodiazepine receptor, peripheral, porin (VDAC) component of; cancer, role of hexokinase and porin (VDAC) in; diabetes mellitus, role of glucokinase in; glucokinase, role in diabetes mellitus; glycerol kinase, porin (VDAC) binding of; hexokinase, porin (VDAC) binding of; ischemia, cerebral, effect on hexokinase binding; porin, mammalian, the voltage-dependent anion channel (VDAC); tumorigenesis, role of hexokinase and porin (VDAC) in; voltage-dependent anion channel (VDAC), mammalian porin.

THE PORIN-KINASE MICROCOMPARTMENTATION MODEL

The principle role of the mitochondrion is energy metabolism. However, within this global role of generating energy from individual substrates, there are a myriad of specific steps, including transport and catalytic processes. Just as there is a functional organization of these specific steps, there is also a spatial organization of the protein complexes mediating these processes. This spatial organization represents the functional microcompartmentation of energy metabolism within the organellar compartment delimited by the mitochondrion.

In 1983 we proposed a model involving the interaction of hexokinase and glycerol kinase with porin, the pore-forming protein of the outer mitochondrial membrane, at contact sites between the inner and outer mitochondrial membranes (McCabe, 1983). We suggested that these contact sites would be formed by apposition of the porin of the outer membrane with the adenine nucleotide translocator of the inner membrane, and would form a conduit for delivery of matrix-generated ATP to the kinases bound to the outer membrane at the mitochondrial–cytoplasmic interface and for return to the matrix of the ADP generated in the kinase reactions. There was clear evidence that hexokinase (Fieck *et al.*, 1982; Linden *et al.*, 1982) and glycerol kinase (Fieck *et al.*, 1982; Ostlund *et al.*, 1983) could bind to porin, and, in fact, porin had been determined to be identical to the "hexokinase binding protein" (Fieck *et al.*, 1982; Linden *et al.*, 1982). Contacts between the inner and

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outer mitocondrial membranes had been observed by electron microscopy (Hackenbrock, 1968). In addition, it had been observed that the ATP utilized by mitochondrial bound hexokinase was derived predominantly from the mitochondrial matrix rather than from the cytoplasm (Bessman *et al.*, 1978; Gots *et al.*, 1972; Gots and Bessman, 1974; Inui and Ishibashi, 1979). The model that we proposed provided a means for explaining these observations, and subsequent data have confirmed the features of this model (Adams *et al.*, 1991a).

MAMMALIAN PORINS AND HEXOKINASES

The mammalian porins are also known as the voltage-dependent anion channels (VDACs). Porin is found not only associated with mitochondria (Linden *et al.*, 1984), but has also been reported in plasma membranes (Thinnes *et al.*, 1989; Kayser *et al.*, 1989). The pore protein permits movement of the adenine nucleotides across the outer mitochondrial membrane (Roos *et al.*, 1982; Fieck *et al.*, 1982). There are at least four VDAC loci in the human genome, VDAC1–4 (Blachly-Dyson *et al.*, 1993; Ha *et al.*, 1993; Blachly-Dyson *et al.*, 1994). VDAC1 has been mapped to Xq13–21 by fluorescence *in situ* hybridization (Blachly-Dyson *et al.*, 1994), and is the only VDAC for which a function has been elucidated. When VDAC1 is expressed in a strain of yeast without endogenous porin, rat brain hexokinase will bind to the mitochondria, in contrast to those that are transformed with VDAC2 (Blachly-Dyson *et al.*, 1993). The other forms of porin have been mapped as follows: VDAC2 to chromosome 21; VDAC3 to chromosome 12; and VDAC4 to chromosome 1, probably interval 1q24-25 (Blachly-Dyson *et al.*, 1994). While sequence similarity is observed among all four of these VDACs, the portion of the VDAC2 sequence that is available indicates that this form differs more significantly from the other three more closely related forms, and VDAC3 may be a pseudogene (Blachly-Dyson *et al.*, 1994). Another isoform, referred to as HUMPORIN is more closely related to VDAC2, and it remains to be determined whether HUMPORIN is coded by the same or a different locus (Ha *et al.*, 1993).

Hexokinase catalyzes the initial step in glucose metabolism, the phosphorylation of glucose to glucose 6-phosphate. Four isozymes of hexokinase have been described, HK1–4. HK1–3 all have molecular weights of approximately 100 kD and Michaelis–Menten constants (K_M) in the range of 10^{-4}–10^{-6}. HK4, also known as glucokinase, differs from the other isozymes, with a molecular weight of about 50 kD and K_M of approximately 10^{-2}. The difference in molecular weights for these two groups of isozymes is due to an apparent duplication–fusion event involving a 50 kD protoenzyme, which resulted in the formation of HK1–3, with the amino-terminal half of the initial duplicated–fused enzyme evolving a regulatory role, while the carboxy-terminal half retained catalytic function (Nishi *et al.*, 1988; Griffin *et al.*, 1991). The data indicate that HK4 is not a direct descendant of the 50 kD protoenzyme, but rather is the result of deletion of the amino-terminal half of a duplicated–fused intermediate. HK1 is observed ubiquitously in tissues, but is found at highest specific activity in brain and kidney, where it is bound to mitochondria (Katzen and Schimke, 1965; Arora and Pedersen, 1988). HK2 is found in insulin-responsive tissues such as skeletal muscle, adipose tissue, and mammary gland, and mitochondrial binding in these tissues is dependent on the presence of insulin (Katzen and Schimke, 1965; Walters and McClean, 1968; Borrebaek and Spydevold, 1969; Bessman, 1972). HK3 is observed in spleen and liver, and binds to the mitochondrion (Katzen and Schimke, 1965; Katzen, 1967; Adams *et al.*, 1991a). HK4 is present in the liver and the beta cells of the pancreatic islets, and is mitochondrial bound in the pancreas but not in the liver, although in the liver it has been observed in the nucleus (Katzen and Schimke, 1965; Katzen, 1967; Malaisse-Lagae and Malaisse, 1988; Miwa *et al.*, 1990; Andreone *et al.*, 1989; Magnuson *et al.*, 1989). The tissue-specific differences in HK4 binding appear to result from differential splicing of the mRNA coding for the amino-terminus (Koranyi *et al.*, 1992), the portion of the enzyme known to be necessary and sufficient for binding of HK1 to its mitochondrial outer membrane receptor (Gelb *et al.*, 1992).

HEXOKINASE AND CANCER

The possibility of a role for hexokinase and its mitochondrial binding in tumor cell transformation and cancer cell metabolism has been considered for a long time (McCabe, 1983; Adams *et al.*, 1991a; Adams and McCabe, 1994). Elevated hexokinase

activity and increased mitochondrial bound hexokinase have been observed in hepatoma cells (Parry and Pedersen, 1983; Nelson and Kabir, 1986; Bustamante *et al.*, 1981; Denis-Pouxviel *et al.*, 1987). In one rodent tumor cell line, AS-30D cells, the mitochondrially bound hexokinase activity is threefold elevated when compared with brain, the tissue with the highest activity for this enzyme (Arora and Pedersen, 1988). It has been suggested that the increase in hexokinase activity observed in tumor cells may be responsible for the increased glycolytic rate observed as a characteristic of these cells, even when they are in the presence of adequate oxygen (Adams *et al.*, 1991a).

Hexokinase that is bound to mitochondria has preferential access to ATP generated within the mitochondrial matrix (Wilson, 1978, 1980; McCabe, 1983; McCabe and Seltzer, 1986; Adams *et al.*, 1991a). This enzyme binding may have an influence on rapidly proliferating tumor tissues that extends beyond energy metabolism, since glucose 6-phosphate, the product of the hexokinase reaction, is a precursor, via the pentose phosphate pathway, for the nucleotides utilized in DNA and RNA synthesis, as well as for the lipids required for membrane synthesis through the triose phosphates including glycerol 3-phosphate (Arora and Pedersen, 1988). Energy, DNA, RNA, and membrane production are all necessary in substantial quantities for active cell division and rapid tissue growth, such as is observed in cancer.

The mechanism of hexokinase binding in tumor cells remains a matter of speculation (Golshani, 1992). HK1 binds to mitochondria in normal tissues and this isoenzyme is observed in some tumor cells. HK2 binding is insulin responsive (see below) and this isoenzyme is observed in other tumor cells, where it is possible that insulin or insulin-like growth factors are important for mitochondrial binding. These issues are of interest, for if bound hexokinase is important to tumor proliferation, then interrupting this binding could be one approach to cancer treatment. Similarly, blocking the increase in hexokinase activity in tumors, through antisense or other strategies, must be considered a potential adjunct strategy for reducing the rapid growth of tumor tissue.

HEXOKINASE AND DIABETES MELLITUS

Hexokinase has a role in the end organ response to insulin, and recent evidence indicates that hexokinase also is involved in the release of insulin by the beta cells of the pancreatic islets. Mitochondrial binding of this enzyme is implicated in both of these phenomena.

As noted previously, HK2 is present in insulin-sensitive tissues (Katzen and Schimke, 1965). Anti-insulin antibodies cause release of hexokinase from rat mammary gland mitochondria (Walters and McClean, 1968). Insulin increases mitochondrial hexokinase in rat epididymal fat pad (Borrebaek and Spydevold, 1969), and in rat muscle the mitochondrial binding of the HK2 isoenzyme is specifically increased in the presence of insulin (Bessman, 1972). These as well as other investigations indicate that the binding of hexokinase to the outer mitochondrial membrane is influenced by insulin. Just as mitochondrial binding of hexokinase appears to play an important part in the rapid growth of cancers, there is clear indication of the role for this binding in the anabolic processes associated with the normal response to insulin, as well as the apparent disruption of this binding in the catabolic processes associated with diabetes mellitus.

Recently, compelling evidence has accumulated that HK4 is critical for insulin release by the beta cell. We would propose that the mitochondrial binding of HK4 in the pancreatic islets (Malaisse-Lagae and Malaisse, 1988) is an important component of this process. The argument that HK4 is involved in the signal transduction mechanism culminating in insulin release is based on the premise that islet cell glucose metabolism would be a part of the intracellular signalling pathway (Matschinsky, 1990). Rate-limiting steps in glucose metabolism would therefore be important sites on which to focus attention, and hexokinase is recognized as one of the rate-limiting enzymes in glycolysis. The K_M for islet cell HK4 of approximately 10^{-2} is in the physiological range for blood glucose. Since HK4 is rate limiting and catalyzes a first-order reaction, glucose phosphorylation increases in direct response to the blood glucose concentration. Therefore, HK4 meets the criteria for a blood glucose sensor.

Since mutations in HK4 are associated with a specific form of diabetes mellitus, this enzyme must be involved in insulin release (Adams and McCabe, 1994). In the Spring of 1992 two groups reported linkage betwen the HK4 gene and maturity onset diabetes of the young (MODY) (Froguel *et al.*, 1992; Hattersley *et al.*, 1992). Direct involvement of the HK4 gene was demonstrated by identification of a point mutation in the HK4 coding sequence that

converted the glutamate at position 279 to a stop codon (Vionnet *et al.*, 1992). This report tracked the mutation through a pedigree in which MODY was inherited in an autosomal dominant fashion, and showed that an individual with non-insulin dependent diabetes, who had appeared to be an exception to the HK4 linkage, did not evidence this point mutation. Her phenotype differed from her relatives with MODY, and the investigators concluded that she represented an individual with diabetes due to another cause. Accumulation of family data confirmed linkage between MODY and HK4 with a lod score of 23.9, but indicated that heterogeneity was likely with linkage projected in 60% (range 35–80%) of families (Froguel *et al.*, 1993). This report showed no linkage between HK4 and late-onset non-insulin-dependent diabetes mellitus (lod $= -7.73$, $\Theta = 0$). Sixteen HK4 mutations were identified in 18 of 32 families examined with MODY, and lod scores were negative in the remaining 14 families. No HK4 mutations were identified in 21 families with late-onset non-insulin-dependent diabetes mellitus, and none of these families exhibited a positive lod score.

HK4 mutations also are associated with gestational diabetes (Stoffel *et al.*, 1993). Since gestational diabetes had been noted previously in families with HK4 mutations, this group of investigators screened a cohort of 40 women with gestational diabetes who also had a first degree relative with non-insulin-dependent diabetes mellitus. Two individuals were identified with HK4 mutations among these 40 subjects. The investigators projected the prevalence of HK4-deficient diabetes to be approximately 1/2400 in the USA.

Insulin secretory profiles were evaluated in HK4-deficient patients and were clearly abnormal (Velho *et al.*, 1992). During hyperglycemic glucose clamp, reduced beta cell secretory response was observed with decreased plasma insulin and C-peptide, and a profile that differed from that seen in individuals with MODY and normal HK4 activity, as well as those with late-onset non-insulin-dependent diabetes mellitus. Among the HK4-deficient patients, fasting plasma insulin and C-peptide were lower than appropriate for the plasma glucose concentration. During hyperinsulinemic euglycemic (5 mM) glucose clamp, endogenous secretion of insulin was lower in individuals with HK4 deficiency than in controls. This group of investigators speculated that HK4 deficiency raises the plasma glucose threshold for insulin secretion, resulting in chronic hyperglycemia. The accuracy of this speculation was reinforced by work in a transgenic mouse model in which there was increased expression of hexokinase (Epstein *et al.*, 1993). The mouse was generated by pronuclear injection of a construct consisting of the yeast hexokinase B (HKB) coding sequence driven by the rat insulin promoter. Expression of hexokinase activity was increased twofold in the islet beta cells of the mice in this transgenic family. These transgenic mice showed increased serum insulin associated with decreased blood glucose to 50–80% of the control values. Isolated islets from the mice evidenced increased insulin release in response to glucose. These investigations indicated that hexokinase has a key role in the transduction pathway for insulin release.

The naturally occurring HK4 mutations in patients with MODY have permitted structure–function analysis of this enzyme after expression in *E. coli* (Gidh-Jain *et al.*, 1993). The mutations mapped primarily to two regions of HK4: within the active site and contiguous region; and in a region distant from the active site that is predicted to undergo conformational change upon binding of the substrates, resulting in closure of the active-site cleft.

These observations indicate that HK4 has a role in the release of insulin by the pancreatic beta cells. It is also clear from patients with mitochondrial DNA (mDNA) mutations and phenotypes that include diabetes mellitus (Piccolo *et al.*, 1989; Zupanc *et al.*, 1991; Majander *et al.*, 1991; Quade *et al.*, 1992; Ballinger *et al.*, 1992; van den Ouweland *et al.*, 1992; Reardon *et al.*, 1992; Bu and Rotter, 1993; Superti-Furga *et al.*, 1993; Onisihi *et al.*, 1993; Rotig *et al.*, 1993; Gerbitz *et al.*, 1993; Remes *et al.*, 1993; Oka *et al.*, 1993), that mitochondrial energy metabolism is involved in insulin release. We would speculate that mitochondrial binding of HK4, a feature that differentiates the pancreatic from the hepatic form of HK4, is important in the transduction pathway which leads from the sensation of the blood glucose to the release of insulin.

OTHER CLINICAL DISORDERS ASSOCIATED WITH HEXOKINASE ABNORMALITIES

The dependence of the brain on blood glucose for energy metabolism, and the high activity of mitochondrial bound HK1 in this tissue, indicates the

importance of this enzyme and its microcompartmentation for central nervous system function. The effects of reduced glucose or oxygen on the binding of hexokinase to the mitochondria in the brain have been investigated. As reviewed previously, early results have been somewhat contradictory, with hypoglycemia and ischemia resulting in increased bound hexokinase, but hypoxia was reported to increase soluble brain hexokinase (McCabe, 1983). When anoxia was induced terminally in rats by decapitation and the proportions of hexokinase in the mitochondrial and the soluble fractions were measured and compared with controls, the proportion of the hexokinase activity bound to the mitochondria increased (Wilson, 1985). Subsequently we have begun to explore this system using a model that relates more closely to a number of clinical situations, involving ischemia and reperfusion of the brains of neonatal piglets (unpublished data). The results in the cortex during the ischemic phase were very similar to those observed in the reports of ischemic and anoxic brains, with an increase in mitochondrial hexokinase activity. During reperfusion there was a return toward the baseline control level of binding, but the baseline levels were not achieved. A number of unanswered questions remain regarding the impact of ischemia and reperfusion on the functional activity of hexokinase and the changes in glucose metabolism that accompany the alterations in microcompartmentation of this enzyme.

Inborn errors of metabolism involving hexokinase deficiency have been described and recently reviewed (Adams *et al.*, 1991a; Adams and McCabe, 1994). The classical form of hexokinase deficiency presents as a nonspherocytic hemolytic anemia with no other reported phenotypic features. However, one patient has been described who had only a skeletal myopathy in association with this enzyme deficiency. No mutations have been described in any of the patients with hexokinase deficiency.

ADDITIONAL FEATURES OF HEXOKINASE WITH POTENTIAL RELEVANCE TO THE PORIN-KINASE MICROCOMPARTMENTATION MODEL

Evidence from studies of the HK1 protein after proteolytic cleavage indicated that the N-terminal amino acids were involved in the mitochondrial binding of the enzyme (Polakis and Wilson, 1985). Comparison of the amino acid sequences for the N-termini of mammalian HK1 showed that the first 15 amino acids were identical in the human, rat, mouse, and bovine enzymes (Griffin *et al.*, 1991). Therefore, we carried out investigations to determine if these N-terminal 15 amino acids were required for specific binding to the outer mitochondrial membrane (Gelb *et al.*, 1992). A construct, pCMVHKCAT, was generated which contained a chimeric reporter gene construct, consisting of the coding sequence for the N-terminal 15 amino acids of mammalian HK1 coupled to the chloramphenicol acetyl transferase *c*DNA, using the CMV promoter. When expressed in a hepatoma cell line in which the native hexokinase is highly bound to the mitochondria, the HKCAT protein was also bound to the mitochondria. The HKCAT protein interacted specifically with the outer mitochondrial membrane receptor for hexokinase, since purified rat brain HK1 that was added to the incubation mixtures was capable of blocking the binding of HKCAT to the mitochondria and maintaining HKCAT in the supernatant. Expression of a series of constructs that deleted increasing portions of the N-terminal HK1 sequence from the chimeric reporter gene indicated that, of the initial 15 amino acids, residues 2–9 are required for binding of HK1 to its outer mitochondrial membrane receptor. We concluded that the N-terminal amino acids of HK1 are necessary and sufficient for the specific interaction of this enzyme with the mitochondria.

Examination of the N-terminal amino acid sequence for HK1 indicates that it forms an alpha helix. One lateral face of the N-terminal alpha helix of HK1 is extremely hydrophobic and could interact with a hydrophobic region of the VDAC1 sequence and/or with other membrane proteins or lipids associated with VDAC1 (Fig. 1). It remains to be determined whether specific residues are involved in the interaction of HK1 with its receptor, or whether this interaction relies solely on the hydrophobic nature of this alpha helical domain.

Autophosphorylation of hexokinase has been described and could play a role in the regulation of the binding of this enzyme to porin (Adams *et al.*, 1991b; unpublished data). After identifying a protein kinase consensus sequence that was conserved in the C-terminal catalytic halves of the mammalian HK1 enzymes (Griffin *et al.*, 1991), we showed that rat brain HK1 was capable of autophosphorylation and

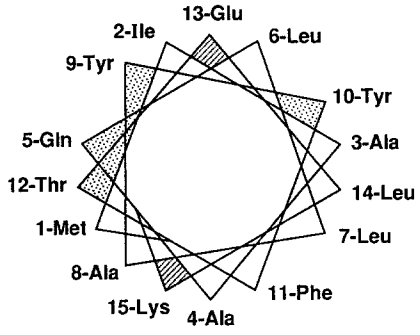

Fig. 1. N-terminal alpha helix of HK1. The N-terminal 15 amino acids that are identical in human, rat, mouse, and bovine HK1 sequences form an alpha helix. They are depicted here as an alpha helical wheel with hydrophobic residues unshaded, charged amino acids shown with diagonal lines, and polar amino acids stippled. One lateral face of this alpha helix (to the right in this figure) is extremely hydrophobic and is completely hydrophobic through the first two turns in the helix; in fact, there is only one amino acid (glutamine 5) within the first two turns that is not hydrophobic.

phosphorylation of a model protein substrate (Adams *et al.*, 1991b). HK1 appears to be a tyrosine kinase (Arora and Pedersen, 1993; unpublished data), as well as a serine/threonine kinase (unpublished data), indicating that it is a member of the new class of dual-specificity protein kinases (Lindberg *et al.*, 1992). Rat brain HK1 is phosphorylated on tyrosine residues in its native state, suggesting a physiologic role for phosphorylation and/or autophosphorylation (unpublished data). Interestingly, the site of tyrosine phosphorylation predicted by computer analysis (Arora and Pedersen, 1993; unpublished data) is near the junction of the N-terminal alpha helical domain with the globular regulatory domain of HK1, a position that could be important for regulation of HK1 binding to the mitochondrial outer membrane receptor by a conformational change of the HK1 that might influence its relationship with the pore protein.

"BALL AND CHAIN" MODEL OF HEXOKINASE–PORIN INTERACTION

Based on the possibility of a dynamic relationship between the bound HK1 and the VDAC1 pore, we proposed a "ball and chain" model to suggest that conformational changes in the hexokinase protein would alter the flux of molecules through the pore (Adams and McCabe, 1994) (Fig. 2). This model is analogous to that which has been proposed for certain ion channels (Jan and Jan, 1989), including the *Shaker* potassium channel (Barinaga, 1990; Hoshi *et al.*, 1990; Zagotta *et al.*, 1990) and the cystic fibrosis transmembrane regulator (CFTR) protein (Kerem *et al.*, 1989).

We suggested that ATP would be involved, possibly through protein phosphorylation, in the conformational change of hexokinase that would regulate flux through the pore protein (Adams and McCabe, 1994). The porin–hexokinase complex in the presence or absence of ADP shows decreased voltage dependency compared with porin alone, when examined in artificial membranes (Brdiczka and Wicker, 1994; unpublished data). However, in the presence of ATP, the porin–hexokinase complex evidences increased voltage dependency compared with porin alone. These investigators have concluded that hexokinase modulates the channel activity of porin, and the modulation of this complex is influenced by the presence of ATP. It is not yet known whether ATP interacts with this complex by non-covalent association and/or protein phosphorylation.

Brdiczka and his colleagues speculate that in the intact cell under physiological conditions porin alone is relatively impermeable to the adenine nucleotides (Gellerich *et al.*, 1993; unpublished data). They suggest, however, that, when the porin–hexokinase complex is formed and ATP binds to this complex, the pore opens up for movement of adenine nucleotides across the outer mitochondrial membrane (Fig. 2). According to this model the porin-hexokinase complex is a dynamic system, the permeability of which is modulated by the presence of its substrate, ATP.

GLYCEROL KINASE: ANOTHER PORIN BINDING PROTEIN

Glycerol kinase binds to porin in varying proportions depending on the developmental stage and metabolic state of the individual tissues (McCabe, 1983; Adams *et al.*, 1991a). The highest proportion bound is observed in rat brain, and, whereas glycerol kinase is highly bound in human fetal liver, this enzyme is predominantly cytoplasmic in livers from infants and adults (McCabe, 1983; Sadava *et al.*, 1987). The glycerol kinase *c*DNA has been cloned (Sargent *et al.*, 1993; Walker *et al.*, 1993; Guo *et al.*, 1993), but investigations like those with HK1 (Gelb *et al.*, 1992), to identify the portion of the protein that interacts with the mitochondrial receptor, have not yet been performed, nor is it known as this time whether mammalian glycerol kinase binds to

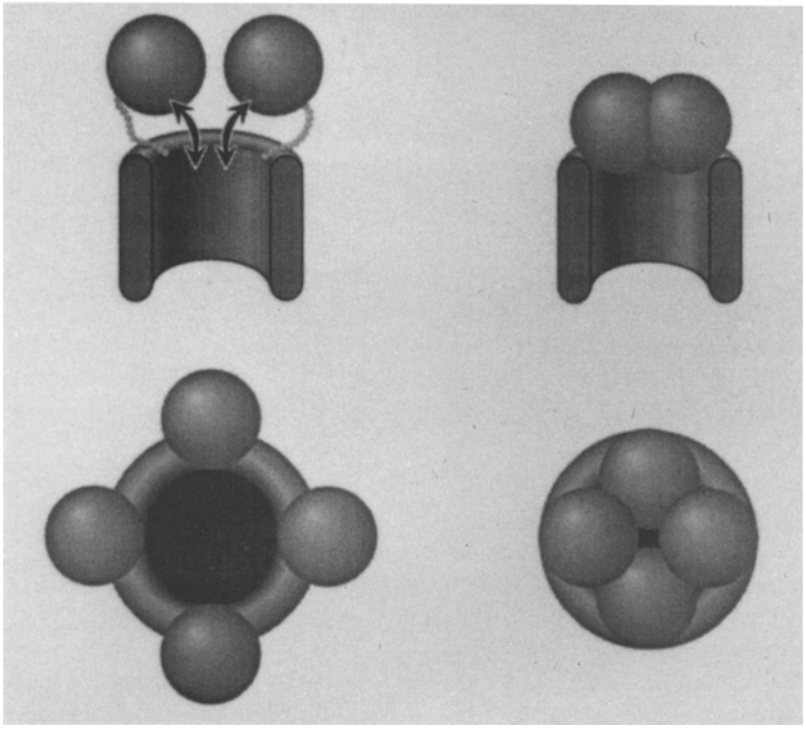

Fig. 2. Model for regulation of porin conductance by hexokinase. This is a "ball and chain" model, in which four hexokinase "balls" are attached to the pore through the "chains," possibly the N-terminal alpha helices. The cartoons on the left show a cut-away side view (top) and an overhead view (bottom) of the hexokinase–porin complex in its open configuration in the presence of ATP. The cartoons on the right show the same views of this complex in its closed configuration in the absence of ATP. (Adapted from Adams and McCabe, 1994.)

VDAC1 (Blachly-Dyson *et al.*, 1993), the form that binds HK1, or to another VDAC protein.

Inherited deficiencies of glycerol kinase map to Xp21 and are categorized into three forms (McCabe, 1994). The infantile form, also known as complex glycerol kinase deficiency, is a contiguous gene syndrome involving deletion of the adrenal hypoplasia congenita (AHC), Duchenne muscular dystrophy (DMD), and/or ornithine transcarbamylase (OTC) loci along with the glycerol kinase (GK) locus. The juvenile form, also referred to as the symptomatic form of isolated glycerol kinase deficiency, has been described in four individuals, two presenting with vomiting and stupor at 4 years of age, one presenting with a Reye-like illness and one with episodic hypothermia and lethargy beginning in the neonatal period. Patients with contiguous gene syndromes involving the GK locus also have eposides of acidemia that may be associated with vomiting and progressive central nervous system depression, even when their adrenal insufficiency is under good control. The adult or benign form of isolated glycerol kinase deficiency is ascertained incidentally in adults without any symptoms attributable to the enzyme deficiency. All patients with contiguous gene syndromes involving the GK locus have demonstrable DNA deletions, but no detectable deletions have been observed among those with the isolated deficiencies, and these patients are presumed to have point mutations, small deletions or duplications, or other rearrangements.

The three-dimensional crystal structure for *E. coli* glycerol kinase has been solved (Hurley *et al.*, 1993). The deduced amino acid sequence for the human glycerol kinase that maps to Xp21 is 50% identical to this prokaryotic enzyme, with an overall similarity of 65%; the key residues involved in binding of the glycerol and adenine nucleotide substrates are nearly completely conserved between man and *E. coli* (Guo *et al.*, 1993). Therefore, point mutations and other perturbations of protein sequence in patients with the isolated deficiencies will be able to be mapped with respect to the functional domains of this enzyme.

Availability of the GK *c*DNA may eventually help us to understand the role of this enzyme in normal central nervous system function. Since glycerol kinase is mitochondrial bound in brain, it is assumed that this normal function involves interaction of this enzyme with porin.

PERIPHERAL BENZODIAZEPINE RECEPTOR: INDEPENDENT CONFIRMATION OF THE FUNCTIONAL INTERACTION BETWEEN PORIN AND THE ADENINE NUCLEOTIDE TRANSLOCATOR

The peripheral benzodiazepine receptor is a mitochondrial structure that is found outside of the central nervous system and appears to be involved in cholesterol uptake by the mitochondrion for steroidogenesis in the adrenal cortex (Papadopoulos, 1993; Lin *et al.*, 1993), as well as in mitochondrial respiration (Hirsch *et al.*, 1989; Moreno-Sanchez *et al.*, 1991). The mitochondrial benzodiazepine receptor consists of a complex of three proteins, with molecular weights of 32, 30, and 18 kD (McEnery *et al.*, 1992). The 32 kD component was identified as one of the VDAC proteins by specific binding of the porin inhibitor, DCCD, and of porin antibodies, and the 30 kD protein was identified as the adenine nucleotide translocator. The 18 kD component, which binds benzodiazepine and is specific to this receptor complex, has been cloned and characterized (Lin *et al.*, 1993). These investigations indicate that porin and the adenine nucleotide translocator are components of the mitochondrial benzodiazepine receptor, and that a third component, the 18 kD benzodiazepine binding protein, is an integral part of the functional receptor complex.

These observations provide independent confirmation of the existence of a complex between porin, and the adenine nucleotide translocator, at the contacts between the mitochondrial membranes, and, hence, of the model that we proposed initially in 1983. This work on the peripheral benzodiazepine receptor suggests a role for proteins (e.g., the 18 kD protein), in addition to hexokinase and glycerol kinase, in the modulation of the conductance at the mitochondrial membrane contact sites. Therefore, ligand-mediated conformational changes in adenine nucleotide flux through the porin-adenine nucleotide translocator complex may be a more general mechanism for signal tranduction.

REFERENCES

Adams, V., and McCabe, E. R. B. (1994). In *Proceedings of NATO ARW, Molecular Biology of Mitochondrial Transport Systems* (Colombini, M., and Fore, M., eds.), Springer-Verlag, Berlin, in press.

Adams, V., Griffin, L., Towbin, J., Gelb, B., Worley, K., and McCabe, E. R. B. (1991a). *Biochem. Med. Metab. Biol.* **45,** 271–291.

Adams, V., Griffin, L. D., Gelb, B. D., and McCabe, E. R. B. (1991b). *Biochem. Biophys. Res. Commun.* **177,** 1101–1106.

Andreone, T., Printz, R. L., Pilkis, S. J., Magnuson, M. A., and Granner, D. K. (1989). *J. Biol. Chem.* **264,** 363–369.

Arora, K. K., and Pedersen, P. L. (1988). *J. Biol. Chem.* **263,** 17422–17428.

Arora, K. K., and Pedersen, P. L. (1993). *Arch. Biochem. Biophys.* **304,** 515–518.

Ballinger, S. W., Shoffner, J. M., Hedaya, E. V., Trounce, I., Polak, M. A., Koontz, D. A., and Wallace, D. C. (1992). *Nature Genet.* **1,** 11–15.

Barinaga, M. (1990). *Science* **250,** 506–507.

Bessman, S. P. (1972). *Isr. J. Med. Sci.* **8,** 344–351.

Bessman, S. P., Borrebaek, B., Geiger, P. J., and Ben-Or, S. (1978). In *Microenvironments and Metabolic Compartmentation* (Srere, P. A., and Estabrook, R. W., eds.), Academic Press, New York, pp. 111–128.

Blachly-Dyson, E., Zambronicz, E. B., Yu, W. H., Adams, V., McCabe, E. R. B., Adelman, J., Colombini, M., and Forte, M. (1993). *J. Biol. Chem.* **268,** 1835–1841.

Blachly-Dyson, E., Baldini, A., Litt, M., McCabe, E. R. B., and Forte, M. (1994). *Genomics*, in press.

Borrebaek, B., and Spydevold, O. (1969). *Diabetologia* **5,** 42–43.

Brdiczka, D., and Wicker, U. (1994). In *Proceedings of NATO ARW, Molecular Biology of Mitochondrial Transport Systems* (Colombini, M., and Forte, M., eds.), Springer-Verlag, Berlin, in press.

Bu, X., and Rotter, J. I. (1993). *Lancet* **342,** 598–600.

Bustamante, E., Morris, H. P., and Pedersen, P. L. (1981). *J. Biol. Chem.* **256,** 8699–8704.

Denis-Pouxviel, C., Riesinger, I., Buehler, C., Brdiczka, D., and Murat, J. C. (1987). *Biochim. Biophys. Acta* **902,** 335–348.

Epstein, P., Boschero, A., Atwater, I., Cai, X., and Overbeek, P. (1993). *Proc. Natl. Acad. Sci. USA* **89,** 12038–12042.

Fieck, C., Benz, R., Roos, N., and Brdiczka, D. (1982). *Biochim. Biophys. Acta* **688,** 429–440.

Froguel, P., Vaxillaire, M., Sun, F., Velho, G., Zouali, H., Butel, M. O., Lesage, S., Vionnet, N., Clément, K., Fougerousse, F., Tanizawa, Y., Weissenbach, J., Beckmann, J. S., Lathrop, G. M., Pass, P., Permutt, M. A., and Cohen, D. (1992). *Nature (London)* **356,** 162–164.

Froguel, P., Zouali, H., Vionnet, N., Velho, G., Vaxillaire, M., Sun, F., Lesage, S., Stoffel, M., Takeda, J., Passa, P., Permutt, A., Beckmann, J., Bell, G., and Cohen, D. (1993). *N. Engl. J. Med.* **328,** 697–702.

Gelb, B. D., Adams, V., Jones, S. N., Griffin, L. D., MacGregor, G. R., and McCabe, E. R. B. (1992). *Proc. Natl. Acad. Sci. USA* **89,** 202–206.

Gellerich, F. N., Wagner, M., Kapischke, M., Wicker, U., and Brdiczka, D. (1993). *Biochim. Biophys. Acta* **1142,** 217–227.

Gerbitz, K. D., Paprotta, A., Jaksch, M., Zierz, S., and Drechsel, J. (1993). *FEBS Lett.* **321,** 194–196.

Gidh-Jain, M., Takeda, J., Xu, L. Z., Lange, A. J., Vionnet, N., Stoffel, M., Froguel, P., Velho, G., Sun, F., Cohen, D., Patel, P., Lo, Y.-M. D., Hattersley, A. T., Luthman, H., Wedell, A., St. Charles, R., Harrison, R. W., Weber, I. T., Bell, G. I., and Pilkis, S. J. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 1932–1936.

Golshani, S. (1992). *Biochem. Med. Metab. Biol.* **47,** 108–115.

Gots, R. E., and Bessman, S. P. (1974). *Arch. Biochem. Biophys.* **163,** 7–14.
Gots, R. E., Gorin, F. A., and Bessman, S. P. (1972). *Biochem. Biophys. Res. Commun.* **49,** 1249–1255.
Griffin, L. D., Gelb, B. D., Wheeler, D. A., Davison, D., Adams, V., and McCabe, E. R. B. (1991). *Genomics* **11,** 1014–1024.
Guo, W., Worley, K., Adams, V., Mason, J., Sylvester-Jackson, D., Zhang, Y.-H., Towbin, J. A., Fogt, D. D., Madu, S., Wheeler, D. A., and McCabe, E. R. B. (1993). *Nature Genet.* **4,** 367–372.
Ha, H., Hajek, P., Bedwell, D., and Burrows, P. (1993). *J. Biol. Chem.* **268,** 12143–12149.
Hackenbrock, C. R. (1968). *Proc. Natl. Acad. Sci. USA* **61,** 598–605.
Hattersley, A. T., Turner, R. C., Permutt, M. A., Patel, P., Tanizawa, Y., Chiu, K. C., O'Rahilly, S., Watkins, P. J., and Wainscoat, J. S. (1992). *Lancet* **339,** 1307–1310.
Hirsch, J. D., Beyer, C. F., Malkowitz, L., Beer, B., and Blume, A. J. (1989). *Mol. Pharmacol.* **35,** 157–163.
Hoshi, T., Zagotta, W. N., and Aldrich, R. W. (1990). *Science* **250,** 533–538.
Hurley, J. H., Faber, H. R., Worthylake, D., Meadow, N. D., Roseman, S., Pettigrew, D. W., and Remington, S. J. (1993). *Science* **259,** 673–677.
Inui, M., and Ishibashi, S. (1979). *J. Biochem.* **85,** 1151–1156.
Jan, L. Y., and Jan, Y. N. (1989). *Cell* **56,** 13–25.
Katzen, H. M. (1967). *Adv. Enzyme Regul.* **5,** 335–356.
Katzen, H. M., and Schimke, R. T. (1965). *Proc. Natl. Acad. Sci. USA* **54,** 1218–1225.
Kayser, H., Kratzin, H. D., Thinnes, F. P., Göetz, H., Schmidt, W. E., Eckart, K., and Hilschmann, N. (1989). *Biol. Chem. Hoppe-Seyler* **370,** 1265–1278.
Kerem, B., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., Buchwald, M., and Tsui, L.-C. (1989). *Science* **245,** 1073–1080.
Koranyi, L. I., Tanizawa, Y., Welling, C. M., Rabin, D. U., and Permutt, M. A. (1992). *Diabetes* **41,** 807–811.
Lin, D., Chang, Y. J., Strauss, J. F., III, and Miller, W. L. (1993). *Genomics* **18,** 643–650.
Lindberg, R. A., Quinn, A. M., and Hunter, T. (1992). *Trends Biochem. Sci.* **17,** 114–119.
Linden, M., Gellerfors, P., and Nelson, B. D. (1982). *FEBS Lett.* **141,** 189–192.
Linden, M., Andersson, G., Gellerfors, P., and Nelson, D. B. (1984). *Biochim. Biophys. Acta* **770,** 93–96.
Magnuson, M. A., Andreone, T. L., Printz, R. L., Koch, S., and Granner, D. K. (1989). *Proc. Natl. Acad. Sci. USA* **86,** 4838–4842.
Majander, A., Suomalainen, A., Vettenranta, K., Sariola, H., Perkkio, M., Holmberg, C., and Pihko, H. (1991). *Pediatr. Res.* **30,** 327–330.
Malaisse-Lagae, F., and Malaisse, W. J. (1988). *Biochem. Med. Metab. Biol.* **39,** 80–89.
Matschinsky, F. M. (1990). *Diabetes* **39,** 647–652.
McCabe, E. R. B. (1983). *Biochem. Med.* **30,** 215–230.
McCabe, E. R. B., and Seltzer, W. K. (1986). In *Myocardial and Skeletal Muscle Bioenergetics: Proceedings of 2nd International Congress on Myocardial and Cellular Bioenergetics and Compartmentation* (Brautbar, N., ed.), Plenum Press, New York, pp. 481–494.
McCabe, E. R. B. (1994). In *The Metabolic Basis of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds.), McGraw-Hill, New York, in press.
McEnery, M. W., Snowman, A. M., Trifiletti, R. R., and Snyder, S. H. (1992). *Proc. Natl. Acad. Sci. USA* **89,** 3170–3174.
Miwa, I., Mitsuyama, S., Toyoda, Y., Nonogakii, T., Aoki, S., and Okuda, J. (1990). *Biochem. Int.* **22,** 759–767.
Moreno-Sanchez, R., Hogue, B. A., Bravo, C., Newman, A. H., Basile, A. S., and Chiang, P. K. (1991). *Biochem. Pharmacol.* **41,** 1479–1484.
Nelson, B. D., and Kabir, F. (1986). *Biochemie* **68,** 407–415.
Nishi, S., Seino, S., and Bell, G. I. (1988). *Biochem. Biophys. Res. Commun.* **157,** 937–943.
Oka, Y., Katagiri, H., Yazaki, Y., Murase, T., and Kobayashi, T. (1993). *Lancet* **342,** 527–528.
Onisihi, H., Inoue, K., Osaka, H., Kimura, S., Nagatomo, H., Hanihara, T., Kawamoto, S., Okuda, K., Yamada, Y., and Kosaka, K. (1993). *J. Neurol. Sci.* **114,** 205–208.
Ostlund, A. K., Gohring, U., Krause, J., and Brdiczka, D. (1983). *Biochem. Med.* **30,** 231–245.
Papadopoulos, V. (1993). *Endocrinol. Rev.* **14,** 222–240.
Parry, D. M., and Pedersen, P. L. (1983). *J. Biol. Chem.* **258,** 10904–10912.
Piccolo, G., Aschei, M., Ricordi, A., Banfi, P., Lo Curto, F., and Fratino, P. (1989). *J. Neurol. Sci.* **94,** 163–172.
Polakis, P. G., and Wilson, J. E. (1985). *Arch. Biochem. Biophys.* **236,** 328–337.
Quade, A., Zierz, S., and Klingmuller, D. (1992). *Clin. Invest.* **70,** 396–402.
Reardon, W., Ross, R. J., Sweeney, M. G., Luxon, L. M., Pembrey, M. E., Harding, A. E., and Trembath, R. C. (1992). *Lancet* **340,** 1376–1379.
Remes, A. M., Majama, K., Herva, R., and Hassinen, I. E. (1993). *Neurology* **43,** 1015–1020.
Roos, N., Benz, R., and Brdiczka, D. (1982). *Biochim. Biophys. Acta* **685,** 202–214.
Rotig, A., Cormier, V., Chatelain, P., Francois, R., Saudubray, J. M., Rustin, P., and Munnich, A. (1993). *J. Clin. Invest.* **91,** 1095–1098.
Sadava, D., Depper, M., Gilbert, M., Bernard, B., and McCabe, E. R. B. (1987). *Biol. Neonate* **52,** 26–32.
Sargent, C. A., Affara, N. A., Bentley, E., Pelmear, A., Bailey, D. M. D., Davey, P., Dow, D., Leversha, M., Aplin, H., Besley, G. T. N., and Ferguson-Smith, M. A. (1993). *Hum. Mol. Genet.* **2,** 97–106.
Stoffel, M., Bell, K., Blackburn, C., Powell, K., Seo, T., Takeda, J., Vionnet, N., Xiang, K. -S., Gidh-Jain, M., Pilkis, S., Ober, C. and Bell, G. (1993). *Diabetes* **42,** 937–940.
Superti-Furga, A., Schoenle, E., Tuchschmid, P., Caduff, R., Sabato, V., DeMattia, D., Gitzelmann, R. and Steinmann, B. (1993). *Eur. J. Pediatr.* **152,** 44–50.
Thinnes, F. P., Goetz, H., Kayser, H., Benz, R., R., Schmidt, W. E., Kratzin, H. D. and Hilschmann, N. (1989) *Biol. Chem. Hoppe-Seyler* **370,** 1253–1264.
van den Ouweland, L. M., Lemkes, H. H., Ruitenbeek, W., Sandkuijl, L. A., de Viklder, M. F., Struyvenberg, P. A., van de Kamp, J. J. and Maassen, J. A. (1992). *Nature Genet.* **1,** 368–371.
Velho, G., Froguel, P., Clement, K., Pueyo, M., Rakotoambinina, B., Zouali, H., Passa, P., Cohen, D. and Robert, J. -J. (1992). *Lancet* **340,** 444–448.
Vionnet, N., Stoffel, M., Takeda, J., Yasuda, K., Bell, G. I., Zouali, H., Lesage, S., Velho, G., Iris, F., Pass, P., Froguel, P. and Cohen, D. (1992). *Nature* **356,** 721–722.
Walker, A. P., Muscatelli, F. and Monaco, A. P. (1993). *Hum. Mol. Genet.* **2,** 107–114.
Walters, E. and McClean, P. (1968). *Biochem. J.* **109,** 737–741.
Wilson, J. E. (1978). *Trends Biochem. Sci.* **3,** 124–125.
Wilson, J. E. (1980). *Curr. Top. Cell. Regul.* **16,** 1–54.
Wilson, J. E. (1985). In *Regulation of Carbohydrate Metabolism*, Vol. I (Beitner, R., ed.), CRC Press, Cleveland, Ohio, pp. 45–85.
Zagotta, W. N., Hoshi, T., and Aldrich, R. W. (1990). *Science* **250,** 568–571.
Zupanc, M. L., Moraes, C. T., Shanske, S., Langman, C. B., Ciafaloni, E., and DiMauro, S. (1991). *Ann. Neurol.* **29,** 680–683.