Disorders of amino acid transport

S. SEGAL
Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia, and Departments of Pediatrics and Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, U.S.A.

Over the past several decades our concept of the cell membrane and its function has undergone considerable change. The anatomic concept of the cell membrane as an inert lipid envelope delimiting the cell from its external environment has been displaced by the formulation that the membrane is a dynamic structure organized to regulate the entry and exit of hydrophilic substances across its 'lipid sea' as well as the transmission of information by means of hormone receptor interactions. Besides containing a complement of specific enzymes, the membrane possesses carrier mechanisms to obviate the prohibitively high amounts of energy required for a hydrophilic substance to permeate such a lipid barrier. In cells with a specialized transport function, such as those of the intestinal mucosa and proximal renal tubule, the membrane has been further differentiated into a mass of finger-like projections, the microvilli, which greatly increases the absorbing surface. The microvillous or brush-border membrane is thus a clearly defined cell organelle, with the extremely important task of orchestrating the transit of molecules into cells.

The recognition of amino acid transport disorders and the abnormality of membrane function stems from the work of Dent & Rose (1951), who studied the Garrodian inborn error, cystinuria. Their findings, based on renal clearance techniques, indicated that defective transport by the renal tubule is the basis of the cystinuria and dibasic aminoaciduria seen in the disease and demonstrated that the transport process is genetically determined. Since that time other inherited transport disorders have been described (Table 1). As originally demonstrated for cystinuria (Milne et al., 1961), many of these involve the intestinal epithelium as well as the renal tubule, but it is the hyperexcretion of urinary amino acids which leads to detection. From a clinical point of view, disorders of amino acid transport present as aminoacidurias. The only exception is the Blue Diaper Syndrome, which results solely from an intestinal absorptive defect for tryptophan and is characterized by the absorption and excretion of indican derived from intestinal flora. Not shown in Table 1 is the group of disorders associated with the Fanconi syndrome (Roth et al., 1981). The latter results from a global disarray of renal transport mechanisms with evidence of a generalized defect not only in amino acid reabsorption but also in altered sugar, phosphate, urate and electrolyte handling. This syndrome is associated with a number of inherited metabolic diseases such as cystinosis, hereditary fructose intolerance and tyrosinaemia, and in most cases is a secondary phenomenon. Taken as a group, amino acid transport abnormalities are among the most common inherited metabolic disorders in man. In addition to their clinical importance, these disorders have aroused the interest of physiologists, biochemists, cell biologists and 'membranologists'. They have been 'experiments of nature' offering experimental guideposts into the examination of the carrier mechanisms present in the membrane for mediating amino acid transport.

In explaining aminoacidurias, recognition must be made of the transcellular fluxes that occur through a renal tubule cell during the absorptive process. In order to be reclaimed, amino acids must pass from the tubule urine into the cell across the luminal brush-border membrane and move out of the cell through the smooth infolded antiluminal membrane. Although the net directional fluxes are for movement in this direction, there is the possibility of outward flux through the brush-border and, of course, entry from the basolateral side. Information about this process has been gained through a variety of physiological techniques, such as micro-perfusion of tubules in situ as well as in vitro. From a biochemical point of view, there is much recent information on the nature of the two types of membranes, since they can be separated (Kenny & Booth, 1978). From work with isolated membrane vesicles, it is known that the brush-border contains Na+-dependent transport systems for amino acids (Kinne, 1976; Sacktor, 1982) and that the basolateral membrane has a facilitated diffusion transport system without Na+-dependence for most substances (Reynolds et al., 1980). The amino acid transport characteristics of each of these diverse membranes determined the various abnormalities possible which may result in transport disorders. Either membrane may be the site of an abnormality responsible for defective substrate reabsorption.

Human cystinuria is the prototypic disorder of amino acid transport and is perhaps the best studied of these entities (Segal & Thier, 1983). The postulate by Dent & Rose (1951), that there was a renal transport defect of a system shared by cystine, lysine, arginine and ornithine, became open to question as patients with hypercystinuria without dibasic aminoacidurias as well as those with hyperdibasic aminoaciduria without cystinuria were described. Direct confirmation of the hypothesis occurred when cystine uptake by intestinal-mucosa biopsies of cystinuric patients examined in vitro demonstrated an absence of an uptake system for all four amino acids and an interaction of cystine and lysine during uptake by normal mucosa (Thier et al., 1964, 1965; McCarthy et al., 1966). The uptake of lysine and arginine by renal cortical slices of human cystinuric patients has been shown to be defective; however, that of cystine was not (Fox et al., 1964). The explanation for this has been derived from experiments with isolated tubule fragments (Foreman et al., 1980) and brush-border membrane vesicles of the normal rat kidney (Segal et al., 1977; McNamara et al., 1981). In both of these preparations two systems for cystine uptake have been demonstrated, a high-Km process not interactive with dibasic amino acids and a low-Km system shared with dibasic amino acids. The shared system is not demonstrable in renal cortical slices; the reason for this discrepancy is unknown. Only the interactive cystine-lysine transport system is found in rat intestine membrane vesicles (OZEgovIC et al., 1982), which aids in interpreting the easily demonstrable defect in cystine and lysine transport in jejunal biopsies from human cystinuric patients. The data thus far are concordant with the Dent & Rose (1951) hypothesis, and suggest that in cystinuria the low-Km system for cystine transport in brush-border membranes is defective. The involvement of the brush-border has been demonstrated in experiments involving the study of cystine flux across affected intestinal mucosa mounted in a transport chamber. The lack of

<table>
<thead>
<tr>
<th>Disease</th>
<th>Increased urine amino acids</th>
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<tbody>
<tr>
<td>Classical hypercystinuria</td>
<td>Cystine, lysine, ornithine, arginine</td>
</tr>
<tr>
<td>Isolated hypercystinuria</td>
<td>Cystine</td>
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<tr>
<td>Lysinuric protein intolerance</td>
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<td>Hyperdibasicaminoaciduria</td>
<td>Dibasic amino acids</td>
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<td>Hyperlysinuria</td>
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<td>Hartnup disease</td>
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<td>Methionine malabsorption</td>
<td>Methionine, branched-chain amino acids, tyrosine, phenylalanine</td>
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<td>Histidinuria</td>
<td>Histidine</td>
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<tr>
<td>Iminoglycinuria</td>
<td>Glycine, hydroxyproline, proline</td>
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<td>Glycinuria</td>
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<td>Dicarboxylic aminoaciduria</td>
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<td>Hypertaurinuria</td>
<td>Taurol</td>
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<td>Tryptophan malabsorption (Blue Diaper Syndrome)</td>
<td>Indicanuria</td>
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tissue from which brush-border membranes from affected patients could be isolated has not yet permitted a direct demonstration of the defective cystine transport mechanism.

Fig. 1 shows, schematically, a possible membrane carrier explanation for the clinical entities involving cystine and dibasic amino acids. In cystinuria without dibasic aminoaciduria (Brodehl et al., 1967) the cystine-specific carrier could be affected. In classical cystinuria, a defect in the brush-border would involve the shared system, with residual specific carriers being normal. Indeed, high filtered loads of lysine are reabsorbed normally, implying that the high-Kₐ process for this amino acid is intact. In hyperdibasic aminoaciduria (Whelan & Svetr, 1968), there could be a defect in a carrier for the dibasic amino acids alone.

The accumulated transport data concerning cystine do not provide an explanation for the often-observed fact that patients with classic cystinuria may have more cystine in the urine than is filtered at the glomerulus. This evidence for 'cystine secretion' may indicate bidirectional cystine transport from the basolateral side of the cell. An intriguing explanation is that by Griffith (1981), who proposed that the excess cystine is derived from intraluminal breakdown of glutathione and an inability to reabsorb the resulting cystine.

At the membrane level there are several possible explanations for defective transport carrier functions as seen in cystinuria and other disorders. There may be an absence of the carrier protein, or it may be incorrectly inserted into the lipid matrix. There could be altered substrate- or Na⁺-binding sites and, under some circumstances, a lack of energy coupling to the membrane. The latter explanation may be applicable to understanding the global transport defect present in the Fanconi syndrome. The study of the gene products responsible for amino acid transport and of how they are altered in transport disorders is in its infancy. There remain many questions relating to membrane structure and composition and how membranes are synthesized and carrier proteins inserted in them. We have yet to learn about the identity of transport proteins, the genetic regulation of their synthesis and the true nature of the abnormality in inherited transport disorders.

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**The low-density-lipoprotein receptor pathway in familial hypercholesterolaemia: its role in pathogenesis and its response to therapy**

CHRISTOPHER J. PACKARD and JAMES SHEPHERD
University Department of Biochemistry, Royal Infirmary, Glasgow G4 OSF, Scotland, U.K.

Recent investigations of the biochemical defects which give rise to FH* have changed radically our comprehension of the mechanisms involved in the regulation of cholesterol metabolism. In this paper, we attempt to summarize the major developments that have occurred in this field over the last decade by reviewing a wide spectrum of biochemical approaches to the problem, ranging from experiments using subcellular fractions to clinical studies of affected individuals.

Abbreviations: FH, familial hypercholesterolaemia; LD lipoproteins, low-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA.

Familial hypercholesterolaemia: the clinical condition

FH is an autosomal dominant condition (Brown & Goldstein, 1974a, 1975) which is characterized clinically by the presence of subcutaneous or tendinous cholesterol deposits, severe hypercholesterolaemia and a family history of ischaemic heart disease. It is one of the most common serious genetic disorders, occurring in its heterozygous form in the population with a frequency of 1 in 500. The rare homozygote has an extremely poor prognosis, often presenting with symptoms of myocardial ischaemia or infarction in childhood, and rarely surviving to the age of 30 (Khachadurian & Uthman, 1973). The plasma cholesterol concentrations of these individuals are typically 6–7 times normal values, and approximately twice the value seen in the heterozygotes. Ultracentrifugation studies (Fredrickson et al., 1978) performed on plasma from these patients have