Studies on the Basic Defect in Cystic Fibrosis: The Kinin Hypothesis

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Cystic fibrosis (McKusick, 1978) is the commonest in-born error in western populations. Over 4% of the population carry the gene, and one child in 2000 is born with the disease. It is transmitted via the autosomal mode of inheritance so that the sexes are equally affected. Patients suffer from a defective function of exocrine glands, the lungs and pancreas in particular becoming progressively affected. In spite of recent improvements in management and treatment, a high proportion of patients fail to reach maturity, usually succumbing to respiratory infections. In spite of extensive investigations over the last 20 years, the basic defect has not been discovered in any enzyme of intermediary metabolism or lysosomal degradation. However, the activity of many of these enzymes is affected by the widespread nature of the disease symptoms (see, for example, Benke, 1976), which include thickening of mucous secretions and fibrotic changes in blocked exocrine glands. As we came to the conclusion that the basic defect must lie in one of the fundamental processes of exocrine gland function, we decided to study the biochemistry of exocrine secretion, which is still not entirely understood.

EXOCRINE GLAND SECRETION

Exocrine glands have a varied range of specialized functions, but can be classified into those which produce a serous or watery secretion, e.g. sweat, salivary and mammary glands, and those like the lung, pancreas and testis which produce a mucoid, i.e. protein and mucus-rich secretion. However, all exocrine glands share a common secretory mechanism comprising the stages of stimulation, intracellular activity, secretion, reabsorption and efflux (Figure 1). Exocrine glands are innervated, and contain α- and β-adrenergic and cholinergic receptors on the serosal side of the secretory cells (Garrett, 1967). In some glands these three channels of stimulation are on a single type of cell (Selinger, 1975), in others these functions may be divided between different cell types (Munger, 1961).

Stimulation of these receptors results in different intracellular responses. Indeed, in some exocrine glands the roles of the intracellular ‘second messengers’ may be interchanged (Table 1). However, the production of a normal secretion requires the mediation of both adrenergic and cholinergic intracellular mechanisms. This intracellular activity results in the production and extrusion of a primary secretory fluid into the lumen of the gland, and this fluid is isotonic (Ullrich, 1974). Secretion from the acini of the gland is followed by reabsorption in the proximal ducts (Mangos, 1973). This process involves the removal of ions from the primary secretory fluid (in partial exchange for K+ and HCO3−), so that the final excretion is hypotonic. Finally efflux to the exterior occurs. In cystic fibrosis patients, both types of exocrine gland produce abnormal secretions. The serous type have elevated Na+ and Cl− concentrations (Lobeck, 1972), whereas the mucoid type produce thickened secretions due to an elevated concentration of Ca2+ (Potter et al., 1963) which causes precipitation within the ducts and the characteristic blockages found in cystic fibrosis.
Table 1  Glandular responses to stimulation in man (Schramm and Selinger, 1975)

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Noradrenaline</th>
<th>Acetylcholine</th>
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<tbody>
<tr>
<td>Receptor</td>
<td>β-Adrenergic</td>
<td>α-Adrenergic</td>
</tr>
<tr>
<td>Second messenger</td>
<td>cAMP</td>
<td>Ca²⁺</td>
</tr>
<tr>
<td>Gland</td>
<td>(Parotid)</td>
<td>(Pancreas)</td>
</tr>
<tr>
<td>Major response</td>
<td>Enzyme secretion</td>
<td>K⁺ release</td>
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<td></td>
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<td>Enzyme secretion</td>
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Since there is a time lag between the onset of fluid production and the attainment of efficient reabsorption (Sutcliffe et al., 1968), and perfusion of isolated glands with isotonic salt solutions alone does not lead to reabsorption (Mangos, 1973), it has therefore been suggested that the primary secretory fluid contains a reabsorption initiation factor (Mangos, 1973). When this factor reaches the proximal duct epithelial cells it causes ionic transfer of Na⁺, Ca²⁺, and Cl⁻ ions across the membrane against a concentration gradient resulting in re-uptake into ductal cells. Ionic equilibration with the interstitial medium occurs, probably via a membrane-bound ATPase on the basal side of the proximal duct epithelial cells.

We postulate that this reabsorption initiating factor is a kinin (Dann and Blau, 1978), and ascribe this role to the glandular kinin kallidin, for which a function in glandular activity has not been defined. The following scheme shows the biochemical pathways for the production of kallidin:

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Exocrine arginine esterase

Glandular pre-kallikrein → Glandular kallikrein

Low mol. wt. kininogen → kallidin

Reabsorption
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Solid lines, Nustad et al., 1978
Broken lines, Dann and Blau, 1978

The glandular kallikrein system seems to be quite distinct to the plasma system, both in terms of immunological differences and substrate specificity (Webster et al., 1963). Whereas plasma kallikrein produces bradykinin from a kininogen precursor, glandular kallikrein produces kallidin.

Several laboratories have used immunofluorescent techniques to demonstrate that glandular kallikreins are localized in secretory granules within the epithelial cells of the proximal ducts in different types of exocrine glands, where they may occur in an inactive form until activated (Orstavik et al., 1975; Dietl et al., 1978). Kallikrein as well as its activating enzyme both belong to the group of trypsin-like enzymes known as 'arginine esterases' and several groups (Rao and Nadler, 1975; Chan et al., 1977) have shown these to be deficient in patients with cystic fibrosis.

DEFECTS IN EXOCRINE FUNCTION IN CYSTIC FIBROSIS

It is our hypothesis that in cystic fibrosis there is a genetically determined specific deficiency in the activity of either the glandular kallikrein or in its activating enzyme, and that this defect is the primary defect in the disease. We do not think the 'plasma system' (i.e. plasma kallikrein, high molecular weight kininogen and bradykinin) is involved in the defect in cystic fibrosis as fluid production in glands of affected patients seems to be normal (Emrich et al., 1968).

The immediate consequence of either of these defects is the absence of kallidin and the resulting failure of reabsorption. The fact that there may be mutations affecting distinct enzymes could account for the genetic heterogeneity of cystic fibrosis.

A consequence of a defective kallikrein system would be accumulation of a precursor related to the low molecular weight kininogen and we propose that this compound is the ciliary-dyskinesia factor. Not only is this factor found extensively in exocrine secretions of cystic fibrosis patients (Mangos and McSherry, 1968; Lobeck, 1972) but it is capable of inhibiting ion reabsorption in the glands of laboratory animals (Mangos and McSherry, 1968) or of normal human subjects (Kaiser et al., 1970) when administered by retrograde perfusion. This factor, therefore, must possess the necessary structure for binding to ductal cells because it includes the 'kinin sequence', but cannot initiate ion reabsorption due to its larger size and different charge distribution.

We thank the Cystic Fibrosis Trust and the Robert McAlpine Foundation for support.