Cystic Fibrosis: Model of Pathogenesis Based on the Apical Membrane Potential

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REVIEW

Cystic fibrosis: model of pathogenesis based on the apical membrane potential

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ABSTRACT

A simple model of cystic fibrosis (CF) is proposed, based on the apical membrane (ApM) potential. The ApM of epithelial cells is highly permeable to sodium and activation of CFTRs makes it permeable to chloride. Calculated ApM potentials of cells with activated cystic fibrosis transmembrane conductance regulators (CFTRs) are between the sodium and chloride Nernst values and thus allow rapid absorption of both ions in exocrine glands. In CF patients the potential is near the sodium Nernst value and thus more salt is left in the ducts. Simulation predicts that the sodium driving force increases more than 3.5 times if the ApM permeability for Cl- increases from 5-94% of the sodium permeability. In pancreatic ductal cells basolateral sodium bicarbonate cotransporters (pNBC1) allows influx of bicarbonates with sodium. Bicarbonates are exchanged for intraductal chloride by anion exchanger 1 (AE1) in the ApM. Activated CFTRs let some chloride to leak back to ducts, followed by water that dilutes ductal proteins. Replenished intraductal chloride allows more bicarbonate secretion. In CF patients, pancreatic water and bicarbonate secretion is limited by the intraductal chloride pool.

Key words: membrane potential, cystic fibrosis, pNBC1, AE1

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INTRODUCTION

This paper on electrophysiological aspects of cystic fibrosis is based on the previous paper about importance of chloride (Cl^-) membrane traffic and Donnan effect of cytoplasmic proteins (1). Cystic fibrosis (CF) is an inherited disease caused by dysfunctional chloride channels, cystic fibrosis transmembrane conductance regulator (CFTR) in various epithelial cells (2). Since this disease is often diagnosed by measuring increased negativity of the transepithelial potential, calculating the basolateral and apical membrane potentials by using the Goldman's equation calculators seemed suitable for the model of pathogenesis (3,4).

BASIC ASSUMPTIONS BEHIND THE PROPOSED MODEL

The model of cystic fibrosis pathogenesis presented here uses several assumptions.

The CFTR is among other regulators of the ductal pancreatic cell function: anion exchanger 1 (AE1), responsible for the exchange of chloride for bicarbonate (HCO_3^-), sodium-hydrogen antiporter 1 (NHE-1), encoded by the SLC9A1 gene, pNBC1 that imports two bicarbonate ions and one sodium (Na^+) in the cell (5).

Dysfunctional CFTRs in patients with cystic fibrosis affect several organs and tissues. Genes for all these structures of ion traffic are variably expressed in all epithelial cells affected by cystic fibrosis (6,7), but high expressions of CFTR and pNBC1 genes seem unique for pancreas.

Membrane ion traffic is governed by electric fields, concentration gradients and ion specific membrane permeability. All cell membranes act as diffusion bottlenecks and if ions accumulate near the membrane, their electric charges alter diffusion of other ions (5).

If a membrane allows only one ion to diffuse along its concentration gradient the diffusion will continue until the membrane reaches the Nernst value of that ion. Further ion traffic depends on Brownian kinetic that washes away ions near the membrane.

Cell membranes are permeable to more than one ion and the actual membrane potential is calculated by the Goldman equation (3,4). Cells permeable to potassium (K^+) and chloride ions normally have membrane potential somewhere between the respective Nernst values.

An example: the resting potential in neurons is near the potassium Nernst potential and traffic of K^+ is opposed by a strong electric force. Traffic of ions across the membrane is intensive during the action potential.

The apical membrane (ApM) of epithelial cells faces the ductal lumen. Due to presence of ENaCs, ApM is highly permeable to sodium and activation of CFTRs makes it permeable to chloride. Except in some kidney cells, ApMs of various epithelial cells are probably less permeable to potassium than their basolateral membrane (BlM). This means that the actual ApM potential depends mainly on Na^+ and Cl^- entering the cell along their concentration gradients. Since these two ions have very different Nernst values, the expected intermediary membrane potential would allow both ions to enter with ease.

In normal individuals and in CF patients transepithelial potential is negative. Since the outer epithelial surface consists of closely arranged apical membranes, this potential has to be related to the actual potential of apical membranes, although the measured transepithelial potential is probably smaller than the actual ApM potential.

THE PROPOSED ELECTROPHYSIOLOGICAL MODEL OF THE APICAL MEMBRANE POTENTIAL

Table 1 shows model predictions of the apical membrane potentials in cells with and without functional CFTRs. The Goldman's equation calculators (3,4) are used to simulate transepithelial voltage in normal ducts with increased Na permeability through ENaCs, and high Cl^- permeability through activated CFTRs. For the 1st, basolateral column values of the generic cell setting in the calculator (3) were used. Sodium and potassium permeability values were interchanged in the remaining two columns that show the apical membrane potentials. Without functional CFTRs (3rd column with low chloride permeability) the apical membrane potential is so near the sodium Nernst value that diminished sodium traffic depends on Brownian mediated diffusion of cations away from the apical membrane. With functional CFTRs (2nd column with chloride permeability that is 94% of sodium permeability), the apical membrane potential is in the middle between the

Table 1. Simplistic model of ion traffic of epithelial cells of normal individuals and patients with cystic fibrosis, calculated by Goldman's equation (3,4)

Parameters of Goldman's equation		Basolateral membrane	Apical membrane permeability in epithelial cells	
			Epithelium with cAMP activated CFTRs	Epithelium without cAMP activated CFTRs, as in CF patients
Sodium	PNa+	5	100	100
	[Na+]out	145	145	145
	[Na+]in	15	15	15
Potassium	PK+	100	5	5
	[K+]out	4.5	4.5	4.5
	[K+]in	120	120	120
Chloride	PCl-	10	94	5
	[Cl-]out	116	116	116
	[Cl-]in	20	20	20
Nernst potentials (mV)	ENa	+60.6	+60.6	+60.6
	EK	-87.7	-87.7	-87.7
	ECl	-47.0	-47.0	-47.0
Electrochemical driving force (mV)	VDF,Na	-121.09	-54.40	-15.28
	VDF,K	+27.22	+93.91	+133.03
	VDF,Cl	-13.53	+53.16	+92.28
VDF,Na ratio of Na+ traffic: activated CFTRs / non-activated CFTRs				3.56
Goldman's equation results (mV)		-60.5	+6.2	+45.3
Predicted potential between intraductal content & IF (assumed to be 0 mV) IF-(apical-basolateral)			-66.7	-105.8
Comments		both K+ and Cl- easily cross since the basolateral potential is intermediary to their Nernst values	Na+ and Cl- both easily cross since the apical membrane potential is intermediary to their Nernst values	low influx of Na+ due to membrane potential close to the Na+ Nernst value and almost no Cl- or K+ influx due to low permeability

Cl^- and Na^+ Nernst values, so the electric field is allowing the maximal traffic of these two ions. The model predicts that permeability values of sodium and chloride need to be almost equal to allow the maximal influx of these ions through the apical membrane. Predicted potential difference between intraductal content and interstitial fluid (IF) is also calculated. It is assumed that this potential is the source of the measured negative transepithelial potential and, as expected, potential is more negative in epithelial cells with dysfunctional CFTRs.

Apical membranes of epithelial cells are highly permeable to sodium (ENACS), and probably some potassium. Permeability to Cl^- through CFTRs depends on activation by cAMP (5). Chloride that enters through functional CFTRs maintains an intermediary apical membrane potential that allows rapid absorption of both ions (Table 1). In this way, activation of CFTRs modulates salt absorption in various exocrine glands.

Epithelial cells in exocrine glands of patients with cystic fibrosis are in a specific situation. Their apical membranes cannot modulate the Cl^- permeability via CFTRs and only the small potassium permeability keeps the membrane potentials from hitting the sodium Nernst value that would further compromise Na^+ diffusion. When the ApM is compared with the IF potential of 0 mV, a more negative difference can be predicted in these patients (Table 1), leading to more negative intraductal potentials. Altered permeability to chlorine makes the apical membrane potential so high that this reduces sodium transport and more salt is left in the ducts. Based on simulation in Table 1, the sodium driving force increases more than 3.5 times if the apical membrane permeability for Cl^- increases from 5 to 94% of the sodium permeability.

The remaining question is why respiratory tract mucosa is the most damaged epithelial tissue in cystic fibrosis patients when similar transepithelial potentials are found in sweat glands and in salivary glands without much damage. A plausible explanation is that it is a consequence of the failed salt reabsorption from the evaporating fluid that covers respiratory mucosa. Each day a normal adult exhales some 400 mL of pure water, and near one half of it comes from the respiratory airways. The only way to prevent accumulation of hypertonic residues after evaporation is quickly to reabsorb salt before evaporation. This desalinization is done via combined action of ENACs and CFTR in normal individuals. In CF patients this process is compromised and some minerals from 150 to 200 ml of evaporated IF similar fluid

remain on mucosal surfaces after water evaporation. The hypertonic ductal content forces some water to remain in ducts due to osmosis and this salty microenvironment is prone to infection and biofilm formation by respiratory bacteria (8).

The presented model interpretation of CFTR function in pancreatic ductal cells is complex. Basolateral membranes in these calls contain pNBC1 that carries two bicarbonate ions with sodium, and each action allows three osmotically and electrically active particles to enter the ductal cell. Excess sodium is taken away by Na^+/K^+ pumps but the faith of bicarbonates is variable. Bicarbonates can interact with intracellular H^+ ions and form carbonic acid that can interact with carboanhydrase and leave the slightly more alkaline cell as CO_2. Alternatively, bicarbonates can leave the cell in exchange for intraductal Cl^- via AE1 structures in the apical membrane. Due to this trade, the overall sum of chloride and bicarbonates concentrations in the ductal content remain almost the same until secretion of bicarbonates is stimulated through opening of CFTRs in the apical membrane.

The AE1 action is probably bidirectional, as is described in erythrocytes during the chloride shift (5). This means that bicarbonates leave the cell if intraductal Cl^- is higher than the cytoplasmic level. Nevertheless, when the pool of intraductal Cl^- is exhausted, the AE1 stops and both bicarbonate and chloride levels in pancreatic ducts and in ductal cells become similar, meaning that without replenishing of the ductal Cl^- pool, pancreatic juice is limited in the bicarbonate content and thus in the enzymatic activity.

Normally, due to various stimuli, intracellular cAMP opens CFTRs on the apical membrane and some influxed Cl^- leak back to ducts, thus allowing more bicarbonates to be secreted by AE1. The consequence is that the sum of Cl^- and bicarbonates increases during the bicarbonate secretion. This is in concordance with the report that pancreatic secretion in two dogs shows more than a 100-fold variation (9), while concentrations of sodium and of potassium were independent of secretory rates. At low secretory rates, bicarbonate levels dropped to values equal to, or lower than plasma concentration, while the concentration of chloride varied inversely with that of bicarbonate.

It is here proposed that by returning the influxed Cl^- ions, the CFTRs add some water to the ductal content, since Cl^- and bicarbonates are both osmotically active and this added water dilutes proteins secreted by acinar pancreatic cells, allowing the diluted and highly alkaline juice to leave pancreas. In patients with CF, the model predicts that the basal secretion produces juice with limited concentration of bicarbonates until the ductal chloride pool is so exhausted that Cl^- concentration is near the cytoplasmic level. This interpretation suggests that accumulated intracellular excess of bicarbonates cannot leave to the duct due to AE1 function failure, and this can lead to cellular alkalosis, via carboanhydrase action. It is well known that cellular alkalosis increases Donnan effect of cytoplasmic proteins due to increased number of protein-bound charges in alkaline pH. The model predicts that this might block further influx of bicarbonate ions via pNBC1, despite the sodium gradient and increased affinity for cations by the Donnan. This means that the ion traffic across the ductal cell apical membrane would be halted until the ductal chloride pool is replenished by the acinar cells.

This interpretation is in concordance with reports that the defect in agonist-stimulated ductal bicarbonate secretion in patients with CF is predominantly due to decreased NBC-driven bicarbonate entry at the basolateral membrane, in the absence of functional CFTR (10). The consequence is that without CFTRs pancreas lacks cAMP modulated secretion of bicarbonates, chloride and water needed to dilute proteins in the pancreatic juice below the dangerous concentration of enzyme activation within the gland.

POSSIBLE EXTRAPOLATIONS OF THE PRESENTED MODEL

Simplistic electrophysiological model of CF changes in ion traffic in various cells presented here clearly suggest that ion traffic is seriously altered in CF patients who are homozygous for the mutation. The remaining question is what can be expected in heterozygous individuals without evident CF. Their phenotype needs to carry some survival advantage that allowed the CF mutation to spread in European nations. Estimated mutation incidences range from 1:200 in northern Sweden, 1:143 in Lithuanians, 1:38 in Denmark to

high values in Italy, France, Switzerland, British Isles, Germany and Greece (11,12). On the other hand, Saamis and Finnish have the lowest rates in Europe (13), while the highest incidences have been found in some disparate locations such as Ireland, Romania, Slovakia and Bulgaria (14).

It is certain that the overall information is blurred by continuous human migrations during the last 50 Ky. This period is crucial due to the estimate that the most common CF linked mutation is possibly no more than 52 Ky old (15).

The model presented here is possibly related to the CF incidence interpretation proposed by M. Lubinsky (16) that involves complex interactions between climate, pathogens and human physiology. The climate factors that are considered are temperature, latitude and altitude, the probable pathogen is tuberculosis while the physiological mechanisms involve vitamin D availability and arterial hypertension. The basic idea is that altered Cl- transport suppresses tuberculosis and alleviates the risk of hypertension caused by salt ingestion. On the other hand, low vitamin D availability, due to scarce sun exposure, increases the chances for tuberculosis and hypertension. This vitamin D and CFTR mutation link is based on the distribution of CF mutations with a high incidence among people from northern latitudes with low vitamin D levels in food and low insolation, except among Inuits, who have rich vitamin D sea food diet. On the other hand, cold climate, high altitude and vitamin D deficiency can all increase risk of arterial hypertension that becomes a very strong selective pressure during pregnancy. Thus, the heterozygote individuals for the CF mutation might have been more resistant to hypertension in this setting due to increased salt losses.

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Model mehanizma oštećenja u cističnoj fibrozi temeljen na apikalnom membranskom potencijalu duktalnih stanica

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SAŽETAK

U radu je prikazan jednostavni model oštećenja u bolesnika s cističnom fibrozom (CF), temeljen na membranskom potencijalu apikalne membrane (APM) duktalnih stanica koja je normalno vrlo propusna za natrij, a u slučaju aktivacije CFTR kanala postaje propusna za klorid. U modelu izračunati APM potencijali stanica s normalnom aktivnošću CFTR kanala pokazuju vrijednosti između Nernstovog potencijala natrija i Nernstovog potencijala klora, što znači da oba iona prolaze s lakoćom i apsorbiraju se iz vodova egzokrinih žlijezda. U bolesnika s CF-om, izračunati apikalni membranski potencijal je blizu Nernstove vrijednosti za natrij, što znači da električno polje priječi apsorpciju natrija a time i više soli ostaje u vodovima žlijezda. Simulacija predviđa da pokretačka snaga apsorpcije natrija raste više od 3,5 puta, ako se APM propusnost za klor povećava u rasponu od 5% do 94% propusnosti za natrij. U stanica vodova gušterače, bazolateralne membrane sadrže natrij/bikarbonata kotransporter (pNBC1) koji omogućuje prolaz bikarbonata zajedno s natrijem. Bikarbonati se razmjenjuju za intraduktalni klorid preko anionskog izmjenjivača 1 (AE1) na apikalnoj membrani. Aktivni CFTR kanali dozvoljavaju povrat klorida iz stanice nazad u vodove pankreasa. Klorid prati voda koja razrjeđuje sadržaj u vodovima. Recirkuliranje klorida u vodove omogućuje veću sekreciju bikarbonata. U bolesnika s cističnom fibrozom, sekrecija bikarbonata i vode je ograničena intraduktalnom količinom klorida koja se ne obnavlja kroz CFTR.

Ključne riječi: membranski potencijal, cistična fibroza, pNBC1, AE1