PULMONARY EPITHELIAL SODIUM-CHANNEL DYSFUNCTION AND EXCESS AIRWAY LIQUID IN PSEUDOHYPOALDOSTERONISM

Eitan Kerem, M.D., Tzvy Bistritzer, M.D., Aaron Hanukoglu, M.D., Thomas Hofmann, M.D., Zhaoqing Zhou, Ph.D., William Bennett, Ph.D., Eithne MacLaughlin, M.D., Pierre Barker, M.D., Martin Nash, M.D., Lynne Quittell, M.D., Richard Boucher, M.D., and Michael R. Knowles, M.D.

ABSTRACT

Background   Active sodium absorption is the dominant mechanism of ion transport in airway epithelium, but its role in pulmonary physiology and airway host defense is unknown. To address this question, we studied the function of airway epithelial cells and determined the frequency of pulmonary symptoms in patients with systemic pseudohypoaldosteronism, a salt-losing disorder caused by loss-of-function mutations in the genes for the epithelial sodium channel.

Methods   In nine patients 1.5 to 22 years of age who had systemic pseudohypoaldosteronism, we tested for mutations in the genes for the epithelial sodium channel, estimated the rate of sodium transport in the airway, determined the volume and ion composition of airway surface liquid, reviewed clinical features, collected laboratory data pertinent to pulmonary function, and, in three adults, measured mucociliary clearance.

Results   The patients with systemic pseudohypoaldosteronism had loss-of-function mutations in the genes for the epithelial sodium-channel subunits, no sodium absorption from airway surfaces, and a volume of airway surface liquid that was more than twice the normal value. The mean ($\pm$SE) mucociliary transport rate was higher in the 3 adult patients than in 12 normal subjects (2.0 ± 0.7 vs. 0.5 ± 0.3 percent per minute, $P=0.009$). Young patients (those five years of age or less) all had recurrent episodes of chest congestion, coughing, and wheezing, but no airway infections with *Staphylococcus aureus* or *Pseudomonas aeruginosa.* Older patients (those more than five years of age) had less frequent respiratory symptoms.

Conclusions   Patients with systemic pseudohypoaldosteronism fail to absorb liquid from airway surfaces; the result is an increased volume of liquid in the airways. These results demonstrate that sodium transport has a role in regulating the volume of liquid on airway surfaces. (N Engl J Med 1999;341: 156-62.)

PSEUDOHYPOALDOSTERONISM is a rare syndrome characterized by defective sodium transport in the distal nephron and renal salt wasting despite high serum aldosterone concentrations. Two clinically distinct forms of pseudohypoaldosteronism have been described.[1] The systemic form is characterized by salt loss from multiple organs, including the kidneys, colon, salivary glands, and sweat ducts. It is inherited as an autosomal recessive trait and is caused by loss-of-function mutations in the genes for an epithelial sodium channel in those tissues.[2,3] The renal form is characterized by salt loss from the kidneys. It is inherited as an autosomal dominant trait and is caused by mutations in the mineralocorticoid-receptor gene.[4] Both forms of pseudohypoaldosteronism present in the first week of life with dehydration, hyponatremia, and hyperkalemia. Children with systemic, but not renal, pseudohypoaldosteronism have frequent lower respiratory tract illnesses of unknown cause.[5,6]

It is difficult to predict the phenotype and pathophysiology of lung disease in patients in whom airway epithelial sodium transport may be absent. Active sodium absorption is the dominant pathway of ion transport in airway epithelium, but the relations among sodium transport, the volume and composition of airway surface liquid, and the efficiency of mucociliary clearance have not been clearly defined.[7-12] If sodium-channel mutations associated with systemic pseudohypoaldosteronism cause a loss of airway sodium transport, the study of patients with these mutations would provide an opportunity to determine the role of sodium transport in the physiology of airway surface liquid and, possibly, defense against lung disease. Therefore, we studied airway epithelial sodium transport and the volume and composition of airway surface liquid in patients with pseudohypoaldosteronism and characterized their pulmonary syndrome.

TABLE 1. DEMOGRAPHIC CHARACTERISTICS, SWEAT AND SALIVARY ELECTROLYTE CONCENTRATIONS, AND GENOTYPES OF PATIENTS WITH SYSTEMIC PSEUDOHYPOALDOSTERONISM.*

PATIENT NO.	AGE (YR)/ SEX	ETHNIC OR RACIAL BACKGROUND	SWEAT Na^+ AND Cl^-†	SALIVARY Na^+ AND Cl^-‡	MICROBIOLOGIC RESULTS	FEV$_1$	GENOTYPE	
							SUBUNIT	MUTATION
			mmol/liter			% of predicted		
1	1.5/M	Northern European white	99, 127	87, 165	*Haemophilus influenzae*§	ND	α	Arg56Stop (256C→T)
					Moraxella catarrhalis§		α	Deletion of exons 3 and 4
2	3/F	Hispanic	ND, 115	147, 136	ND	ND		Unknown
								Unknown
3¶	6/M	Iranian Jewish	118, 110	146, 140	*Streptococcus pneumoniae*§	85	α	Arg508Stop (1621C→T)
							α	Arg508Stop (1621C→T)
4¶	6/M	Iranian Jewish	130, 110	138, 80	*Streptococcus pneumoniae*§	115	α	Arg508Stop (1621C→T)
							α	Arg508Stop (1621C→T)
5	6/M	Hispanic	ND, 120	118, 111	Normal flora‖	86		Unknown
								Unknown
6	11/F	Iranian Jewish	170, 150	160, 143	Normal flora‖	97	α	Arg508Stop (1621C→T)
							α	Arg508Stop (1621C→T)
7	18/F	Ashkenazi Jewish	100, 100	149, 136	Normal flora‖	92	β	Frame shift (647insA)
							β	Frame shift (915delC)
8	18/M	Hispanic	122, 120	80, 71	Normal flora‖	93	α	Frame shift (604delAC)
							α	Frame shift (1404delC)
9	22/M	Northern European white	111, 141	145, 141	Normal flora‖	110		Unknown
								Unknown

*FEV$_1$ denotes forced expiratory volume in one second, ND not determined, C cytosine, T thymidine, and A adenine.

†Normal sweat sodium and chloride concentrations were <70 mmol per liter and <60 mmol per liter, respectively.

‡Normal salivary sodium and chloride concentrations are <60 mmol per liter.

§Microbiologic results are from culture of bronchoalveolar-lavage specimens.

¶Patients 3 and 4 are dizygotic twin brothers.

‖Microbiologic results are from culture of sputum.

METHODS

Subjects

We studied nine patients from eight families with systemic pseudohypoaldosteronism (Table 1) and four patients from three families with renal pseudohypoaldosteronism (three boys and one girl 6 to 10 years of age). The diagnosis of pseudohypoaldosteronism was based on severe renal salt wasting in early infancy in association with hyponatremia, hyperkalemia, metabolic acidosis, hyperreninemia (plasma renin activity of more than 10 ng per milliliter per hour [2.8 ng per liter per second]), and hyperaldosteronism (serum aldosterone concentrations greater than 100 ng per deciliter [2700 pmol per liter]), but who otherwise had normal adrenal function. The patients with systemic pseudohypoaldosteronism, but not those with renal pseudohypoaldosteronism, had high sodium and chloride concentrations in the saliva and sweat (Table 1). Gastroesophageal reflux had been diagnosed in six of the children 1.5 to 6 years of age with pseudohypoaldosteronism[13]; two had undergone Nissen fundoplication, with no change in respiratory symptoms. Two of the patients (Patients 6 and 9) have previously been described.[2,14] The study was approved by the human-rights committees at all participating centers, and informed consent was obtained from all patients or their parents.

Clinical Evaluation

In all the patients we evaluated respiratory symptoms and performed a physical examination. In most patients, we performed chest and sinus radiography and microbiologic examination of sputum or bronchial-lavage fluid; determined atopic status by skin-prick testing[15]; determined the clinical or spirometric response to an inhaled β-agonist drug or a glucocorticoid; and performed spirometry. In some patients, we measured lung volumes by body plethysmography or functional residual capacity by the nitrogen-washout technique, the diffusing capacity of the lung by the carbon monoxide technique, and bronchial reactivity by methacholine challenge.[16-19]

Detection of Mutations

Genomic DNA isolated from peripheral leukocytes or messenger RNA isolated from nasal epithelium was tested for mutations in the genes encoding the α, β, and γ subunits of the epithelial sodium channel by single-strand conformation polymorphism analysis with the use of products amplified by the polymerase chain reaction[2,20] and by direct-sequencing analysis.

Sodium Transport in the Airway Epithelium

Rates of sodium transport in nasal and bronchial epithelium were estimated by measuring the basal transepithelial voltage and determining the reduction in voltage during luminal perfusion of amiloride (10^{-4} M) or sodium-free Ringer's solution.[21,22]

Volume and Composition of Airway Surface Liquid

Nasal and bronchial surface liquids were collected by the filter-paper technique.[23] In two patients with systemic pseudohypoaldosteronism, approximately 3 ml of clear liquid was aspirated from bronchial surfaces with a transbronchoscopic catheter. Ion composition, volume, and osmolarity $[2([Na^+]+[K^+])]$ were estimated as described previously.[23]

Bronchoscopy

Three patients underwent diagnostic bronchoscopy, and research studies were performed during the procedure. Two patients (both 6 years of age) were sedated with parenteral midazolam, fentanyl, and atropine, and one patient (18 months of age) was sedated with parenteral propofol. Lidocaine (2 percent) was applied only to the pharynx and vocal cords. After the collection of surface liquid, a 3.5-mm bronchoscope (model 20PD or 3C20, Olympus, Lake Success, N.Y.) was wedged in the right lower lobe for bronchoalveolar lavage (three aliquots of 1 ml of normal saline per kilogram of body weight). The lavage fluid was cultured for quantitative determination of bacteria[24] and was examined for cells and protein.[23]

Mucociliary Clearance

In three adults with systemic pseudohypoaldosteronism, clearance rates of technetium-99m–labeled iron oxide particles (median diameter, 4.5 μm) from the lungs were determined by gamma-camera analysis.[25] The initial deposition patterns were determined, and activity in the right lung was monitored for two hours. Clearance rates (percentage cleared per minute) were calculated from a best-fit regression of retained activity against time and compared with values in normal adults.[25]

Statistical Analysis

Values are expressed as means ±SE. Bioelectric and electrolyte values for each nostril were averaged to give a value for each subject. The differences between study groups were compared by nonpaired t-tests. The difference between the mucociliary clearance rates in the normal subjects and in the patients with systemic pseudohypoaldosteronism was evaluated by the Kruskal–Wallis test.[26]

RESULTS

Course of Respiratory Disease

All patients with systemic pseudohypoaldosteronism had salt wasting and electrolyte disturbances 24 to 72 hours after birth, but none had respiratory symptoms. Within weeks or months after birth, all patients had respiratory illnesses (three to six per year) characterized by chest congestion, cough, and tachypnea and often associated with fever, wheezing and crackles, and mild leukocytosis. Blood cultures were negative, and bacterial causes were not identified. In patients more than five years of age, respiratory illnesses were less severe and less frequent. Skin tests were negative for common allergens. No patient had digital clubbing, chronic sinusitis, clinical or radiographic evidence of bronchiectasis, or airway infection with *Staphylococcus aureus* or *Pseudomonas aeruginosa.*

Genotypes in Patients with Systemic Pseudohypoaldosteronism

Three patients, each born to consanguineous parents, were homozygous for a stop mutation (Arg508Stop) resulting from a cytosine-to-thymidine mutation at nucleotide 1621 of the gene for the α subunit of the sodium channel (Table 1). One patient had two frame-shift mutations in the gene for the α subunit (deletion of adenine and cytosine at nucleotide 604 and deletion of cytosine at nucleotide 1404), one patient had two frame-shift muta-
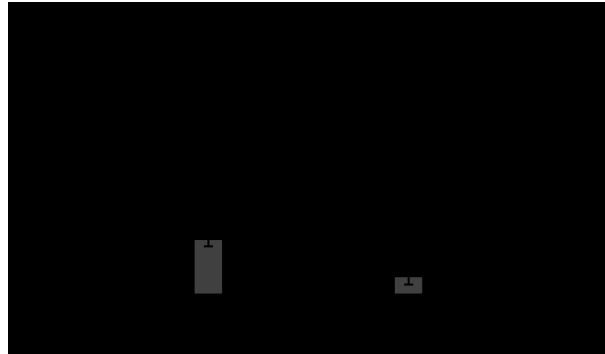

Figure 1. Mean (±SE) Bioelectric Indexes of Nasal Epithelial Sodium Transport in 33 Normal Subjects (Open Bars), 9 Patients with Systemic Pseudohypoaldosteronism (Solid Bars), and 3 Patients with Renal Pseudohypoaldosteronism (Hatched Bars).

The data from the normal subjects (age range, 1.5 to 22 years) were reported previously.[21] Asterisks indicate P<0.001 for the comparison with normal subjects and with patients with renal pseudohypoaldosteronism. Values shown are basal values (before perfusion of the lumen with amiloride) and percent inhibition (after perfusion).

tions in the gene for the β subunit (insertion of adenine at nucleotide 647 and deletion of cytosine at nucleotide 915), and one patient had one stop mutation (Arg56Stop, resulting from a cytosine-to-thymidine mutation at nucleotide 256) and one in-frame deletion (deletion of exons 3 and 4) in the gene for the α subunit.

Bioelectric Measurements in the Airway

Electrogenic nasal epithelial sodium transport was absent in patients with systemic pseudohypoaldosteronism (Fig. 1). The basal transepithelial potential difference was only about 35 percent of the normal value, and the potential difference was not reduced by perfusing the lumen with amiloride, a sodium-channel inhibitor (mean [±SE] change, −0.9±0.4 mV), or with sodium-free Ringer's solution (mean [±SE] change in three patients, +2.7±1.2 mV).[22] The bronchial potential difference measured in one patient (Patient 1) was much lower (−2 mV) than that in normal subjects (approximately −20 mV) and was not reduced by amiloride.[27]

Volume and Composition of Nasal Surface Liquid

The volume of nasal surface liquid obtained from the patients with systemic pseudohypoaldosteronism was more than twice that obtained from normal subjects (Fig. 2). The concentration of sodium in the surface liquid from patients with systemic pseudohypoaldosteronism was higher and that of potassium was lower than in the samples from normal subjects or patients with renal pseudohypoaldosteronism (Fig. 2). The surface liquid was approximately isotonic (about 305 mmol per liter) with plasma.
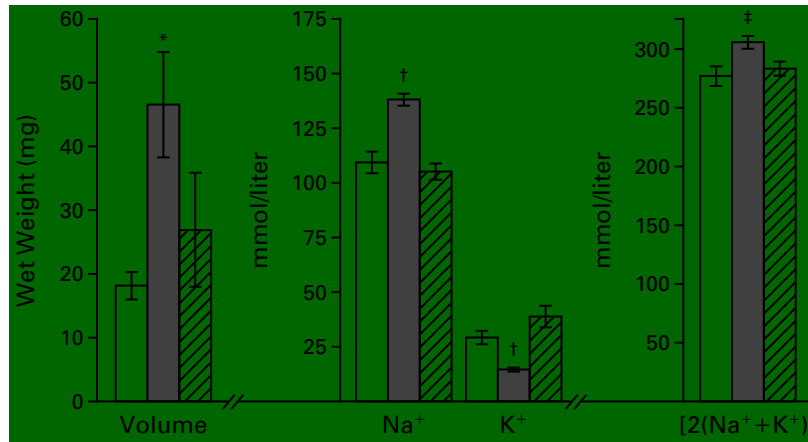
Figure 2. Mean (±SE) Volume for Weight, Ionic Composition, and Estimated Osmolarity [2([NA+]+[K+])] of Nasal Surface Liquid Recovered from Eight Normal Subjects (Open Bars), Eight Patients with Systemic Pseudohypoaldosteronism (Solid Bars), and Three Patients with Renal Pseudohypoaldosteronism (Hatched Bars).

The asterisk indicates P=0.009 for the comparison with normal subjects; the daggers indicate P<0.001 and the double dagger indicates P=0.04 for the comparisons with normal subjects and patients with renal pseudohypoaldosteronism.

Children with systemic pseudohypoaldosteronism had a chronic nasal drip of clear liquid that caused irritation of the philtrum area (Fig. 3A).

Bronchoscopy

The three patients who underwent diagnostic bronchoscopy (Patients 1, 3, and 4) had a large volume of bronchial surface liquid (Fig. 3B). The protein concentration of the liquid was 24 and 40 mg per deciliter (<1 percent of the serum protein concentration) in the two patients in whom it was measured (Patients 3 and 4, respectively), and the mean osmolality was 298 mOsm per kilogram. The concentration of sodium in the liquid was high (141±10 mmol per liter) and that of potassium was low (7.9±3.5 mmol per liter) in comparison with that in normal bronchial surface liquid.[23]

Microbiologic Examination

There were inflammatory cells and bacteria in the bronchial-lavage fluid from the three patients who underwent bronchoscopy. The proportion of neutrophils ranged from 10 to 79 percent of the recovered cells. One patient (Patient 1) had a large amount of cloudy liquid in the proximal airways, from which *Moraxella catarrhalis* and *Haemophilus influenzae* were cultured (100,000 and 40,000 colony-forming units per milliliter, respectively). In two patients (Patients 3 and 4) in whom the liquid was clear, *Streptococcus pneumoniae* was cultured (50,000 and 100,000 colony-forming units per milliliter, respectively). The five older patients (6 to 22 years of age) produced small sputum samples that contained only normal flora.

Pulmonary-Function Tests

The results of spirometry were normal in seven patients who were tested when they were not acutely ill. Of the four children tested, three (Patients 3, 4, and 6) had mild air trapping (increased functional residual capacity or residual volume), whereas the three adults did not. Methacholine-challenge tests were positive in four of the five patients tested (Patients 3, 4, 6, and 9). However, only two of seven patients treated with bronchodilators had improvement on spirometric measurements or clinical benefit, and only one had a clinical response to treatment with inhaled glucocorticoids.

Radiographic Studies

Patients six years of age or less had hyperinflation, apparent bronchial thickening, and intermittent segmental atelectasis, but no evidence of bronchiectasis or lobar consolidation. Chest radiographs in the older patients were normal. Sinus radiographs were normal in seven of eight patients.

Mucociliary Clearance

There was rapid transport of radiotracer in the three patients with systemic pseudohypoaldosteronism in whom we tested mucociliary clearance (Fig. 4). The mean clearance rate in the 3 patients was more rapid than in the 12 normal subjects (2.0±0.7 vs. 0.5±0.3 percent per minute for the initial 20 minutes, P=0.009).[25]

DISCUSSION

The patients with systemic pseudohypoaldosteronism had the typical clinical features of the disor-

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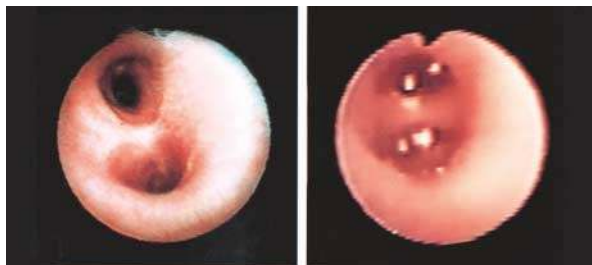
B

Figure 3. Excess Airway Liquid in Patient 3, a Six-Year-Old Boy with Systemic Pseudohypoaldosteronism.

Panel A shows chronic nasal drainage of clear liquid in the absence of viral or allergic rhinitis and the irritation of the philtrum area resulting from the nasal drainage. Panel B shows transbronchoscopic views of the bronchial surfaces in a normal subject, six years of age, who underwent bronchoscopy because of a possible foreign body (left) and the patient with systemic pseudohypoaldosteronism (right).

der, with severe neonatal salt wasting, hyponatremia, hyperkalemia, metabolic acidosis, high serum aldosterone concentrations, and recurrent respiratory illness in the first years of life.[1-3,5] These patients required high doses of salt replacement into adulthood. These clinical features contrasted with those of the patients with renal pseudohypoaldosteronism, in whom the disease was limited to the kidney and who did not require dietary salt supplementation after the age of five years.

We identified frame-shift, deletion, or stop mutations in the genes for the subunits of the epithelial sodium channel on both alleles in six of the nine patients with systemic pseudohypoaldosteronism (Table 1). These mutations cause systemic pseudohypoaldosteronism, probably by eliminating sodium-channel function.[2,3,28] The three patients with no identified

mutations may have intronic or promoter mutations or mutations in other proteins responsible for the assembly or function of sodium channels.

The patients with systemic pseudohypoaldosteronism had a total absence of electrogenic sodium transport in the upper and lower airways (Fig. 1). The chief physiologic consequence of defective sodium transport was an increase in the volume of airway surface liquid. The presence of excessive liquid on airway surfaces was associated with a distinct respiratory syndrome. Children had persistent rhinorrhea of clear liquid, with chronically chapped upper lips. In the first years of life, these children also had recurrent respiratory illnesses characterized by congestion, tachypnea, wheezing, and frequently fever. Although aspects of these illnesses were suggestive of asthma, only two of the patients had high serum IgE concentrations (Patients 7 and 9), and they did not benefit from therapy with aerosolized β-adrenergic agonists or glucocorticoids.

We speculate that the pulmonary syndrome in patients with systemic pseudohypoaldosteronism is caused by an excessive volume of surface liquid that narrows airway lumens and dilutes surface-active materials that stabilize small airways,[29,30] predisposing the patients to wheezing and airway infections early in life. As in viral bronchiolitis, airway narrowing due to intraluminal liquid may be more prominent in infancy and early childhood when the airway diameter is small. This speculation is compatible with the positive results of methacholine challenge in four of the five patients tested; such results can be due to a decreased airway caliber in patients who do not have atopy.[31,32]

The failure to absorb liquid as it moves up the converging surfaces of the airways predicts that sufficient liquid will accumulate on proximal airways to produce airflow obstruction — that is, "intrapulmonary drowning."[7] To compensate for the failure to absorb liquid from airway surfaces in patients with systemic pseudohypoaldosteronism, the rate of mucociliary clearance increases to remove some of the excess volume from airway surfaces. The mechanism of the increased rate of mucociliary clearance in these patients is unclear. The efficiency of mucociliary clearance is thought to be dependent on contact of ciliary tips with the inner surface of a layer of mucus. Our data from patients with systemic pseudohypoaldosteronism suggest that an expansion of the volume of airway surface liquid does not "float" mucus off the tips of cilia and slow mucociliary clearance. It is more likely that the excess volume is added to the mucous layer, improving its biorheologic properties and clearance rates.[7,33]

The functional defects in the airway epithelium of patients with systemic pseudohypoaldosteronism provide insights into the relations among epithelial sodium transport, the volume and composition of air-
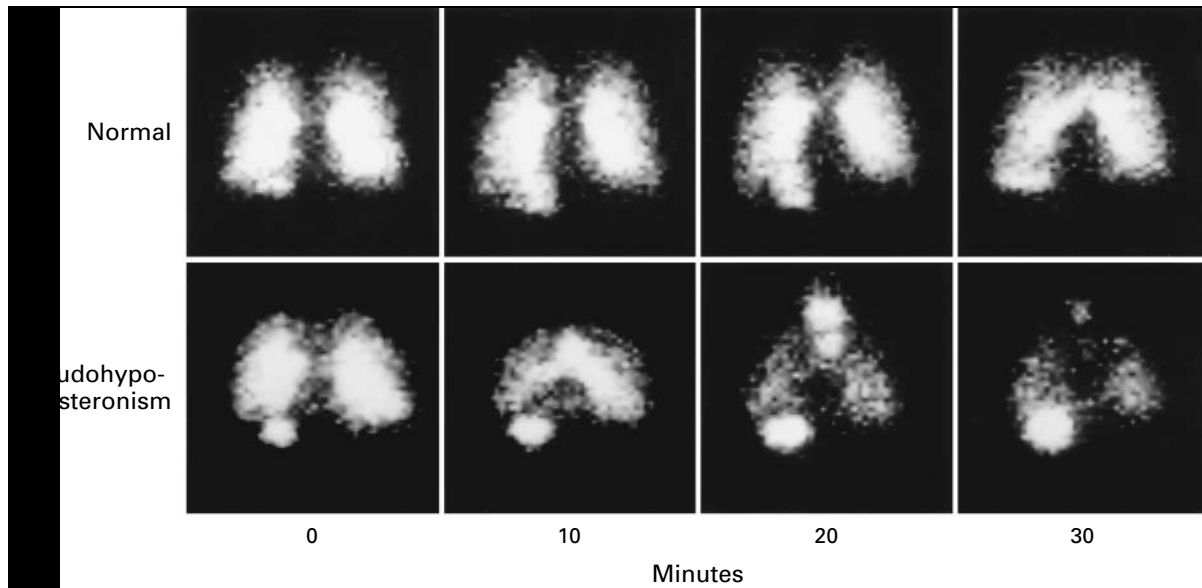
Figure 4. Posterior Gamma-Camera Radionuclide Images of Whole-Lung Clearance in a Normal Subject and a Patient with Systemic Pseudohypoaldosteronism Immediately after Inhalation of Technetium-99m–Labeled Iron Oxide Particles and 10, 20, and 30 Minutes Later.

The distribution of tracer is relatively uniform in both base-line images, but there is rapid clearance of particles into the proximal airways of the patient with pseudohypoaldosteronism (10 minutes). There is a sequential increase in the accumulation of tracer in the stomach (beneath the left lung).

way surface liquid, and the pathogenesis of airway disease, including cystic fibrosis. Some have suggested that the function of sodium transport in the airway epithelium is to generate hypotonic (i.e., low sodium and low chloride) airway surface liquid that promotes the antimicrobial activities of salt-sensitive defensins.[10,11] According to this hypothesis, chronic bacterial airway infections in patients with cystic fibrosis reflect the inability of the airway epithelium to lower the concentration of sodium and chloride in airway surface liquid, thereby inactivating defensins and promoting infection. Others have suggested that sodium transport normally regulates the volume of an isotonic airway surface liquid, and that this regulation is important for mucus clearance.[23,27,33] According to this hypothesis, sodium and volume absorption is increased in cystic fibrosis, mucus transport is abolished, and chronic infection follows.[33] The data from the patients with systemic pseudohypoaldosteronism argue for the latter hypothesis: sodium transport is linked to regulation of the volume of airway surface liquid, not to its tonicity, and chronic bacterial airway infection is not associated with isotonic airway surface liquid if mucus clearance is preserved.

In summary, studies of patients with systemic pseudohypoaldosteronism demonstrate the linkage between sodium transport and the volume of airway surface liquid, rather than its tonicity, in lung physiology. The pulmonary disease in young children with

systemic pseudohypoaldosteronism represents a novel form of airway disease associated with the excess volume of airway surface liquid resulting from defective sodium-dependent liquid absorption. An increase in the rate of clearance of liquid from airway surfaces by mucociliary transport appears to compensate, in part, for the failure to absorb liquid transepithelially.

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