These results with ANF demonstrate that acute renal repair of chronic chloride-depletion metabolic alkalosis in a rat model system can be initiated without replacement of the chloride or potassium deficits (or without pharmacological inhibition of tubular function). They support the thesis that the resolution of chronic metabolic alkalosis can be achieved by normalizing GFR.

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12. I thank J. Lewicki for supplying the ANF, F. Rector, Jr., for ongoing support and advice, and K. Wong and M. Chambers for technical assistance. Supported in part by a Clinical Investigator Award (1-K08-AM 01015) and a grant (AM-27045) from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

2 April 1985; accepted 15 July 1985

Deficient Vasoactive Intestinal Peptide Innervation in the Sweat Glands of Cystic Fibrosis Patients

Abstract. *The innervation of acini and ducts of eccrine sweat glands by immunoreactive, vasoactive intestinal peptide–containing nerve fibers was sharply reduced in seven patients with cystic fibrosis compared to eight normal subjects. The decrease in innervation by this neuropeptide, which has been shown to promote blood flow and the movement of water and chloride across epithelial surfaces in other systems, may be a basic mechanism for the decreased water content and relative impermeability of the epithelium to chloride and other ions that characterize cystic fibrosis.*

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Cystic fibrosis (CF), the most common lethal genetic disease in Caucasians, manifests itself as an exocrinopathy characterized by elevated concentrations of Cl^- and Na^+ in sweat, and other electrolyte abnormalities of exocrine secretions (*1*). The pathogenesis of CF remains poorly understood, but recent investigations have shed new light on the mechanism of the electrolyte disturbance (*2*). In CF, there is an increase in the transepithelial potential difference in sweat gland ducts (*3*) and respiratory mucosa (*4*), which has been attributed to a decreased epithelial permeability to Cl^- (*5*). We report here that CF patients showed a marked deficiency of vasoactive intestinal peptide (VIP)–immunoreactive nerves in both acini and ducts of eccrine sweat glands (*6*). Since VIP promotes blood flow and the movement of water and Cl^- across epithelial surfaces, the deficient VIP innervation may be responsible for the decreased water content and relative impermeability of the epithelium to Cl^- and other ions that characterize CF.

The secretory activity of sweat and other exocrine glands is influenced by adrenergic and cholinergic innervation (*7*) and by the peptidergic system of nerves (*8*). Among the neuropeptides supplying exocrine glands, VIP richly innervates and promotes blood flow to the pancreas and to sweat, salivary, lacrimal, bronchial, and intestinal glands (*9*). This peptide binds to specific membrane receptors in these glands (*10*), stimulates Cl^- and water transport across intestinal (*11*) and tracheobronchial mucosa (*12*), and stimulates HCO_3^- secretion by pancreatic acini (*13*). VIP also reduces the potential difference across the duct of the rabbit submandibular gland (*14*). These observations suggested that VIP exerts a physiologic regulatory influence on exocrine function. Since the effects elicited by VIP are counter to the observed defects in CF, we postulated that the exocrine abnormalities of CF may be caused by a deficiency of VIP innervation. We therefore examined the presence, distribution, and density of VIP-immunoreactive nerves in the sweat glands of normal subjects and CF patients. We selected sweat glands because (i) they reflect one of the principal functional abnormalities of the disease; (ii) unlike other exocrine organs, such as the lungs, they remain free of infection or morphologic changes; and (iii) they are easily accessible through skin biopsy. The study was approved by our Institutional Review Board, and written informed consent was obtained in advance from the patients or their parents.

Skin samples were obtained by biopsy from the chest region in seven CF patients of both sexes, aged 13 to 28 years; control specimens of normal skin (as determined by light microscopy) were removed at mastectomy (from six women aged 36 to 78 years) or leg amputation (from two males aged 7 and 22 years). Skin samples were fixed in picric acid–formaldehyde (*15*) for 3 hours at 4°C, then transferred to 0.1*M* phosphate-buffered saline with 0.3 percent Triton X-100 (PBS-TX) (*p*H 7.8), and rinsed overnight at 4°C. Cryostat sections, 10 μm thick, were incubated for 30 minutes at 37°C with a standardized 40-μl drop of VIP antiserum (rabbit 89N, diluted 1:100 in PBS-TX), washed, and then incubated for 30 minutes at 37°C with goat anti-rabbit immunoglobulin G labeled with fluorescein isothiocyanate (Miles Labs, Elkhart, Indiana). The sections were washed in PBS-TX, mounted on slides in glycerine diluted with PBS-TX (1:4), and examined in a fluorescence microscope (Olympus BH2T) with a mercury vapor lamp.

The specificity of the VIP antiserum in immunocytochemical investigations has been demonstrated (*16*). In our study, specific immunofluorescence was confirmed by absorption of the antiserum with synthetic VIP (100 g per milliliter of diluted antiserum) 24 hours before use and by the substitution of normal rabbit serum for the immune antiserum; no fluorescence was present in either control section.

The extent of VIP innervation to acinar and ductal regions of sweat glands

was rated on a scale of 0 to 5 (Table 1). Three different glands, either in the same section or in three nonserial sections, were examined to determine the rating for each specimen. The rating was assessed by one investigator (R.D.D.) who did not know the origin of the specimens. Statistical comparisons between the two groups were made by the Mann-Whitney *U* test (*17*). Normal skin showed a rich network of VIP-immunoreactive nerves around secretory acini [mean density ± standard error of the mean (SEM), 3.6 ± 0.7] and a moderate innervation of the ducts (1.8 ± 0.9) (Fig. 1). Individual VIP-positive nerve fibers were closely associated with the basement membrane of both acinar and duct cells. VIP innervation in CF samples was considerably less pronounced both in the acini (1.3 ± 1.0) and in the ducts (0.3 ± 0.5) (Fig. 1). VIP innervation was totally absent around the ducts in five of the seven CF samples and was minimal in the remaining two.

The pathogenesis of the exocrinopathy of CF has defied explanation by one unifying hypothesis. A disturbance of autonomic function has been suspected (*18*), but neither cholinergic nor adrenergic mechanisms alone could fully account for the disease manifestations. Recently, neuropeptides such as VIP have been recognized as forming a new component of the autonomic nervous system (*7*). The importance of VIP in the physiology and pathophysiology of exocrine function is suggested by its presence in nerve fibers and nerve terminals close to glandular structures; by its potent influence on blood flow (*9*), macromolecular secretion (*19*), and water and electrolyte transport (*14–16*); and by the identification of specific receptor binding sites for VIP in exocrine organs (*10*).

Our results show that VIP-immunoreactive nerves supply both the secretory acini and the reabsorptive ducts of sweat glands. The close relations between these nerves and the basement membranes provides an anatomic basis for the influence of locally released VIP on the secretion and composition of sweat. If the decreased VIP innervation of CF sweat gland acini and ducts is shown to be a primary abnormality (rather than excessive depletion, for example), it may help to explain the abnormal sweat composition that characterizes those with this disease. CF sweat, sampled by micropuncture at the acinar level, is similar in composition to sweat from normal subjects, the increased Cl^- content in CF sweat resulting from impaired Cl^- reabsorption by the ducts (*20*). The absence or paucity of VIP nerves to sweat gland ducts in CF may be particularly important in this regard, in view of the capacity of VIP to stimulate Cl^- movement (*11, 12*); a deficiency of VIP could reduce Cl^- secretion by acini or its reabsorption by the ducts. A generalized deficiency of VIP innervation of exocrine glands, if confirmed, could also account for other abnormalities in CF, such as decreased pancreatic HCO_3^- secretion. Defective or absent VIP innervation is known to be associated with, and causally related to, pathophysiologic alterations in other organs, including the esophagus in achalasia (*21*) and the gut in Hirschsprung's disease (*22*).

Table 1. Relative frequency of VIP-immunoreactive nerves in the sweat glands of seven CF patients and eight normal control subjects. Frequency of VIP-immunoreactive nerves: none, 0; very few, 1; few, 2; moderate, 3; numerous, 4; very numerous, 5.

Relative frequency	Normal (n = 8)		CF (n = 7)	
	Acini	Ducts	Acini	Ducts
5	1			
4	3			
3	4	2	1	
2		2	1	
1		4	4	2
0			1	5
Mean ± SEM	3.6 ± 0.7	1.8 ± 0.9	1.3 ± 1.0	0.3 ± 0.5

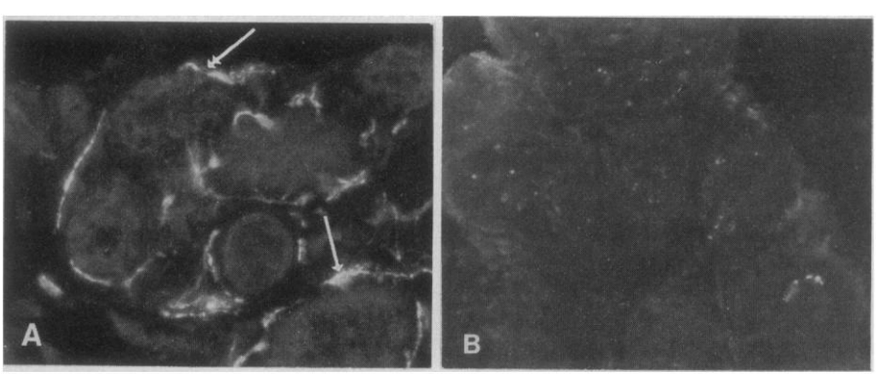

Fig. 1. Cryostat sections of skin from a normal adolescent boy (A) and from a boy of similar age with cystic fibrosis (B). VIP-immunoreactive nerve fibers surround normal sweat gland acini (arrow) and ducts (double arrow); no VIP-containing nerves are seen in the skin section from the boy with cystic fibrosis (×561).

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14 March 1985; accepted 29 July 1985