CHEST

Cystic Fibrosis and the Nervous System

Leah R. Reznikov, PhD

Cystic fibrosis (CF) is a life-shortening autosomal recessive disorder caused by mutations in the gene encoding the *cystic fibrosis transmembrane conductance regulator (CFTR)*. CFTR is an anion channel that conducts bicarbonate and chloride across cell membranes. Although defective anion transport across epithelial cells is accepted as the basic defect in CF, many of the features observed in people with CF and organs affected by CF are modulated by the nervous system. This is of interest because CFTR expression has been reported in both the peripheral and central nervous systems, and it is well known that the transport of anions, such as chloride, greatly modulates neuronal excitability. Thus it is predicted that in CF, lack of CFTR in the nervous system affects neuronal function. Consistent with this prediction, several nervous system abnormalities and nervous system disorders have been described in people with CF and in animal models of CF. The goal of this special feature article is to highlight the expression and function of CFTR in the nervous system. Special emphasis is placed on nervous system abnormalities described in people with CF and in animal models of CF. Finally, features of CF that may be modulated by or attributed to faulty nervous system function are discussed.

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Cystic fibrosis (CF) is one of the most common lethal autosomal recessive genetic disorders seen in the United States.[1] Approximately 1,000 new cases of CF are diagnosed every year.[2] The expectation of life for newborns in the United States born with CF is approximately 40 years.[1]

Mutations in the cystic fibrosis conductance regulator gene (*CFTR*) are responsible for CF.[3-5] CFTR is a member of the adenosine triphosphate (ATP) binding cassette transporters.[6] The channel uses energy from ATP hydrolysis to actively transport anions, such as bicarbonate and chloride, down their concentration gradients. Its structure consists of two membrane-spanning domains, two nucleotide-binding domains, and an unstructured R domain, with a total weight of approximately 170 kDa.[7] The channel activity is regulated by cyclic adenosine monophosphate (cAMP)-dependent phosphorylation of the R domain and hydrolysis of intracellular nucleotides at the nucleotide-binding domains.

Currently, > 1,800 mutations in the *CFTR* gene are known.[1,8] All mutations do not produce the same degree of disease nor do all mutations affect channel function equally.[9] The most common mutation is a three-base pair deletion, resulting in a loss of a

ABBREVIATIONS: ATP = adenosine triphosphate; cAMP = cyclic adenosine monophosphate; CF = cystic fibrosis; CFRD = cystic-fibrosis-related diabetes; CFTR = cystic fibrosis transmembrane conductance regulator

AFFILIATIONS: Department of Physiological Sciences, University of Florida, College of Veterinary Medicine, Gainesville, FL.

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CORRESPONDENCE TO: Leah Reznikov, PhD, Department of Physiological Sciences, University of Florida, College of Veterinary Medicine, 1333 Center Dr, PO Box 100144, Gainesville, FL 32610; e-mail: leahreznikov@ufl.edu

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phenylalanine at position 508. This mutation is responsible for approximately 70% of CF alleles.[5] The ΔF508 mutation impairs CFTR's ability to exit the endoplasmic reticulum, reducing expression at the cell surface.[10] Channels that do make it to the surface open less frequently. A person who is homozygous for the ΔF508 mutation has very little channel activity and subsequently a severe disease phenotype.[11]

Distinguishing features of CF is complex and include salty skin, poor growth, intestinal obstruction and malabsorption, infertility, pancreatic insufficiency, frequent airway infection and inflammation, persistent coughing, adherent and viscous mucus, and difficulty breathing.[1,12] Respiratory disease is the major cause of mortality and morbidity in people with CF.[13] Decades of work suggest that a lack of bicarbonate and chloride transport across epithelia is the cause of CF pathogenesis.[14-20] Those studies are reviewed elsewhere and will not be reviewed here.[12,21-25]

Managing CF often requires individualized treatment and care. Current treatments targeting the CF airway include mucolytic agents, chest physiotherapy, antiviral agents, antibiotics, antiinflammatory drugs, bronchodilators, and lung transplantation.[26,27] Small-molecule therapies that target *CFTR* mutations have also become available recently and have shown great success.[27-29] Several institutions continue to pursue gene therapy to correct and restore normal CFTR function[30-33]

CFTR Expression and Function in the Nervous System

Expression of *CFTR* is developmentally regulated and widespread.[34-37] Although expression in epithelial cells has been studied most, immune cells,[38] fibroblasts,[39] heart cells,[40,41] kidney cells,[42] liver cells,[43] skeletal[44] and smooth muscle cells,[45] chondrocytes,[46] osteoclasts,[47] and neural cells[40,48] all express CFTR. Nonepithelial expression is consistent with work demonstrating that CFTR does not require epithelial cell-specific machinery to form a functional channel.[49]

Expression of CFTR in the nervous system is particularly intriguing because chloride transport is a well-known modulator of neuronal activity.[50] In addition, as highlighted further on, many of the features of CF could manifest due to nervous system dysfunction. McGrath et al[40] were the first to describe CFTR in the nervous system. Subsequent studies by Mulberg et al[51,52] detailed CFTR expression in the rat brain (Fig 1). The expression pattern of CFTR suggested a possible role in regulation of mood and memory, energy homeostasis, olfaction, motor function, respiration, and autonomic control of visceral organs.[51,52] Additional studies found CFTR expression or activity (or both) in rodent dorsal root ganglion neurons,[53] hypothalamic neurons,[54] and motor neurons.[37] In pigs, *CFTR* is expressed in both the peripheral and central nervous systems.[55,56] In humans, CFTR has been identified in parvocellular ganglion,[57] hypothalamic neurons,[58] spinal cord neurons,[59] ganglion cells of the heart,[60] and sympathetic ganglion.[61] Both neuronal and glial cell CFTR expression has been reported in multiple species,[55,62-64] with the exception of humans, in whom only neuronal CFTR has been reported.[48,54]

The function of CFTR in the nervous system is uncertain. Reports of CFTR in the hypothalamus sparked speculations that neural CFTR may be important in maintenance of the neuroendocrine

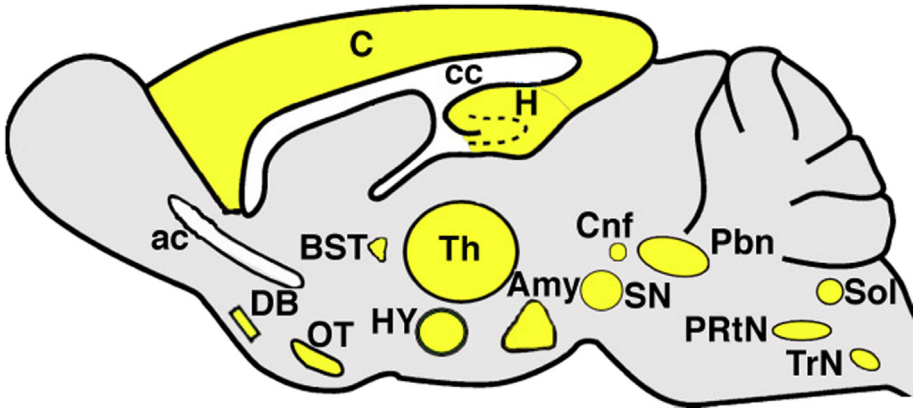

Figure 1 – *CFTR in the rat brain. Summary of work by Mulberg et al[51,52] showing CFTR expression (yellow) in the rat brain. Location suggests involvement in energy homeostasis, limbic function, olfaction, motor function, respiration, and autonomic regulation of numerous effector organs, including the airways, GI tract, and heart. Amy = amygdala; ac = anterior commissure; BST = bed nucleus of stria terminalis; C = cortex; cc = corpus callosum; Cnf = cuneiform nucleus; DB = diagonal band of Brocha; H = hippocampus; HY = hypothalamus; OT = olfactory tubercle; Pbn = parabrachial nucleus, PRtN = parvocellular reticular nucleus; Sol = nucleus of the solitary tract; SN = substantia nigra; Th = thalamus; TrN = trigeminal nucleus.*

axis.[51,52,58] Indeed, decreasing CFTR expression in a rat hypothalamic cell line reduced the release of gonadotropin-releasing hormone.[54] In an additional study, the responsivity of glucose-sensitive neurons in the rat hypothalamus decreased with pharmacologic inhibition of CFTR.[65] Our studies in newborn pigs revealed *CFTR* mRNA in the pituitary and hypothalamus and demonstrated that release of growth hormone is reduced in pituitary slice cultures isolated from CF pigs compared with non-CF littermates.[56] These studies are all consistent with a function of CFTR in modulating the neuroendocrine axis.

A nonneuroendocrine function of CFTR has also been proposed. Ostroumov et al[37] used CFTR inhibitors and computer modeling to demonstrate that CFTR modifies the excitability of motor neurons in the rat spinal cord. The authors went on to speculate that exaggerated neuronal excitability in individuals with CF might contribute to an increased incidence of seizures following lung transplantation.[66] A role for CFTR in modulating ATP release from dorsal root ganglion neurons was reported.[53] Given the important role of ATP in pain responses, it is possible that CFTR in the dorsal root ganglia might modify nociception.[67,68]

An additional set of experiments by Liu et al[63] suggested that microglia isolated from CF mice release less ATP.[63] The implications from this study were that lack of CFTR decreases propagation of calcium waves in microglia, which in turn affects the extension and retraction of microglial processes. The extension and retraction of microglial processes plays an important role in the scavenging of damaged brain tissue.[69] Another study found no obvious role for CFTR in modulating glial cell activity.[64]

Neural Abnormalities in Cystic Fibrosis

Given that CFTR is expressed and functional in the nervous system, it is not surprising that several nervous system abnormalities have been reported in CF. Although it is not known whether these abnormalities are primary or secondary or the extent to which they are manifested in all people with CF, their presence may be a component of, or may contribute to, pathogenesis, or both. Following is a brief summary of selected anomalies.

Innervation

Alterations in neural innervation have been repeatedly reported in CF (Table 1). Heinz-Erian et al[70] were first to describe reduced innervation of the sweat gland in humans with CF. The authors suggested that the reduced innervation of the sweat gland might contribute to the high salt content of sweat in people with CF. A subsequent study also found that cutaneous innervation was decreased in humans with CF.[71] Pan et al[72] described decreased innervation of the CF mouse airway *in utero* that persisted postnatally. The decreased innervation was associated with decreased smooth muscle mass and decreased pulmonary neuroendocrine cells. Interestingly, in humans with CF, the number of pulmonary neuroendocrine cells is elevated.[73,74] Our studies in the pig suggest that innervation of the sinuses[55] and trachea[75] is also decreased. The pattern of innervation in the CF pig pancreas is also qualitatively different and is decreased as assessed by the neural markers β-tubulin III and PGP9.5.[76] No information is available regarding innervation of the intestinal tract in animal models of CF, but hyperplasia of the intestinal nervous system[77] and more prominent nerves[78] has been reported in children with CF. Information regarding innervation of the heart, gallbladder, and reproductive tract is either not known or unavailable. However, abnormalities in neurally controlled functions of the heart,[79,80] gallbladder,[81] and reproductive tract[82] have been reported.

Ventilatory Responses

Abnormalities in ventilation have also been reported in CF. In mice, the ventilatory response to hypoxia is decreased.[83] In people with CF, ventilatory responses to hypoxia are also reduced,[84] whereas exercise causes an exaggerated ventilatory response.[85] Since CF mice do not exhibit lung infections like humans, it seems less likely that a decreased ventilatory response is explained by airway infection or inflammation. Because ventilatory responses involve neural control of both pulmonary and cardiac systems, one interpretation of these findings is that people with CF have dysregulation of the neural cardiopulmonary reflexes. Other factors, such as reduced lung compliance, might also contribute.[86]

Autonomic Function

Several studies suggest that autonomic function is altered in people with CF. Davis and Kaliner[87] found increased pupillary reflexes to cholinergic agonists and α-adrenergic agonists in people with CF. Other studies suggest that people with CF have decreased leukocytic generation of cAMP[88] and impaired cardiac and peripheral hemodynamic responses[89] to β-adrenergic stimulation, as well as exaggerated sweat and saliva secretion in response to cholinergic agonists.[90] Aquagenic wrinkling of the skin is a sympathetic

TABLE 1] Summary of Neural Innervation of Organs Affected in CF and Broadly Related Functions

Major Organ	Innervation Affected in CF	Species	Bodily Function Controlled by Nervous System	Affected in CF	Species
Airways (trachea, bronchus, lungs)	+ Pan et al[72] Reznikov et al[75]	Mouse, pig	Smooth muscle contraction Cough Submucosal gland secretion Respiration	+ Cook et al[45] Reznikov et al[75] + Smith[96] Hamutcu et al[97] Smith et al[98] Chang et al[99] + Sun et al[104] Ianowski et al[107] Joo et al[108] Salinas et al[109] + Bonora et al[83] Bureau et al[84] Bongers et al[85]	Pig, mouse Human Mouse, pig, ferret, human Mouse, human
Intestines	+ Wildhaber et al[77] Collins et al[78]	Human	Gastrointestinal motility	+ Rogers et al[103] Sun et al[104] Zhou et al[105] van der Doef et al[106]	Human, mouse, pig, ferret
Sweat gland	+ Heinz-Erian et al[70]	Human	Sweating	+ Cystic Fibrosis Foundation[1] Wine[25]	Human
Heart	Unknown	. . .	Heart rate	+ Florencio et al[79] Szollosi et al[80]	Human
Pancreas	+ Uc et al[76]	Pig	Glucose regulation	+ Moran et al[111] Marshall et al[112] Kerem et al[113] Moran et al[114] Yi et al[115] Olivier et al[116]	Human, pig, ferret
Gallbladder	Unknown	. . .	Gallbladder emptying	+ Debray et al[81]	Mouse
Reproductive tract	Unknown	. . .	Ovulation	+ Johannesson et al[82]	Human
Eye	Unknown	. . .	Pupillary constriction/ dilatation	+ Davis and Kaliner[87]	Human
Skin	+ Savage et al[71]	Human	Skin wrinkling	+ Grasemann et al[91] Park et al[92] Wilder-Smith[93]	Human

"+" means there are data to support the finding that the innervation or function is affected in CF. References listed directly support the finding that either innervation or bodily function controlled by the nervous system is affected in CF. CF = cystic fibrosis.

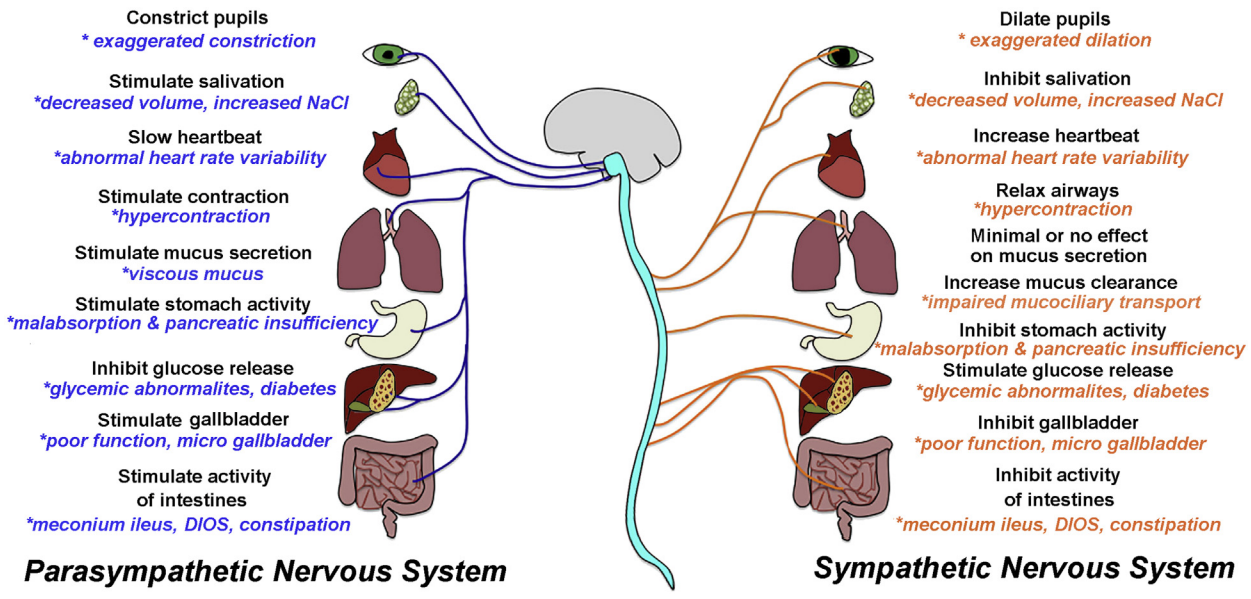

Figure 2 – *Organ dysfunction and the potential impact of neural dysfunction within that organ. Many of the organs affected in cystic fibrosis (CF) are controlled by the autonomic nervous system. Some hallmark features of CF could be modified by, or attributed to, defective neural regulation. Examples of known features and organ functions that are affected in CF are shown with asterisks in blue and orange text. It is important to note that there has been no direct cause and effect relationship established between organ dysfunction and neural dysfunction in CF. DIOS = distal intestinal obstruction syndrome.*

nervous system-mediated response and is also exaggerated in people with CF.[91-93] Alterations in smooth muscle contractile responses to cholinergic agonists have also been described,[45,94] as has abnormal heart rate variability.[79,80] Altered autonomic function could contribute to manifestations of CF, including cough, GI motility, and mucus secretion, topics explored in greater detail further on.

Possible Neural Contributions to CF Pathogenesis

The nervous system exerts widespread control of multiple organs, including those affected in CF (Fig 2). Thus it is possible that nervous system defects could contribute to CF pathogenesis (Fig 3). Select categories of CF features that might be modified by or attributed to (or both) nervous system dysfunction are discussed in the following sections. Additional features are highlighted in Table 1. The following sections are speculative due to limited literature and a lack of clinical studies focused on the nervous system. They are also intentionally brief to minimize diluting relevant points and concepts.

Cough

Cough is a protective behavior mediated by the nerves innervating the airway.[95] The presumed triggers for cough are viscous and adherent mucus, impaired

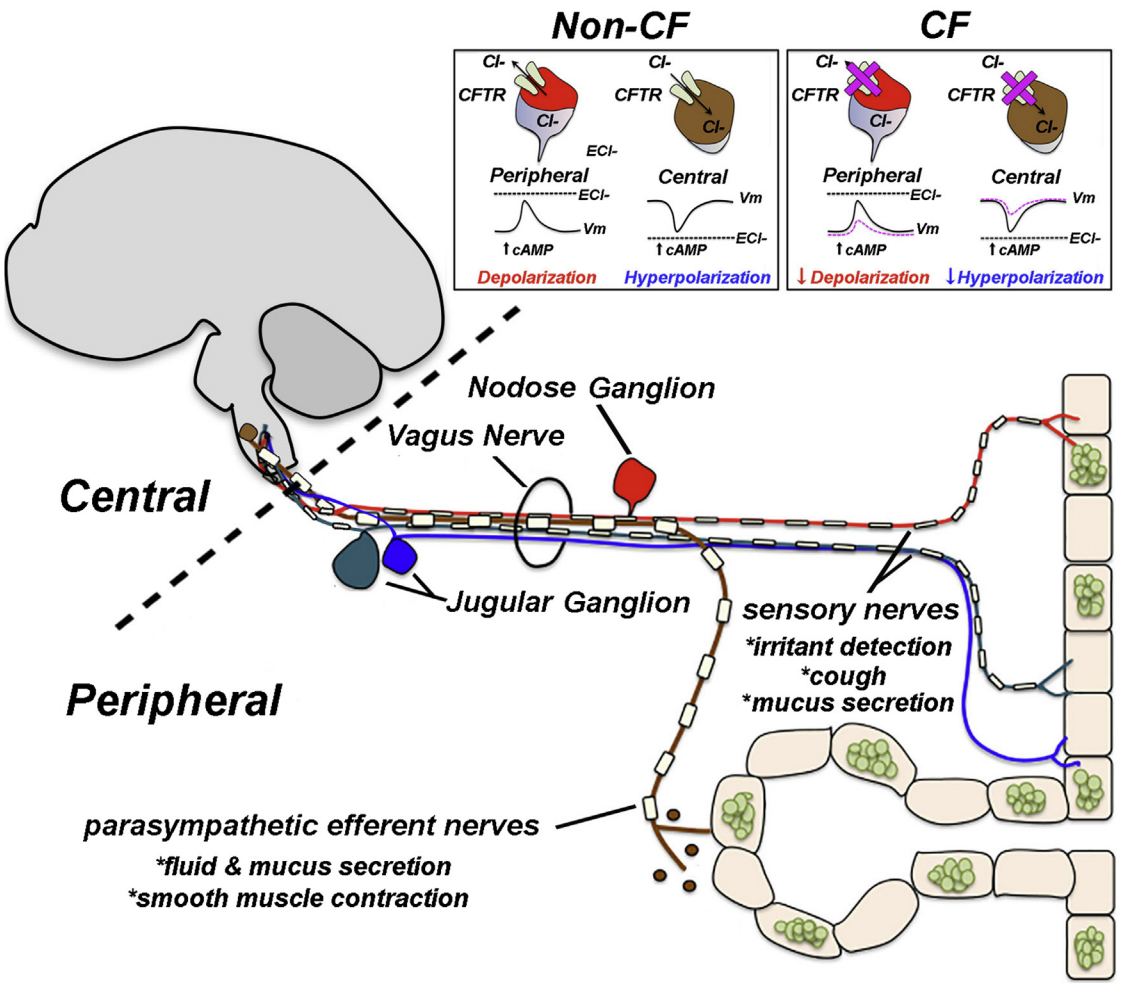

Figure 3 – *Potential mechanism for how loss of CFTR might affect neuronal function and contribute to pathogenesis. Peripheral neurons participate in the detection of irritants and generation of protective reflexes, such as cough. The information is sent to central neurons in the brainstem to cause efferent release of acetylcholine, causing smooth muscle contraction and mucus secretion. In peripheral neurons, chloride (Cl^-) currents are excitatory; therefore, loss of CFTR might decrease peripheral neuron activity. In central neurons, chloride currents are generally inhibitory, and therefore loss of CFTR might enhance central neuron activity. Inset shows hypothetical activation of CFTR by agents that raise intracellular cyclic adenosine monophosphate (cAMP) and predicted outcome on neuronal activity in the absence of CFTR. The asterisks (*) and text identify functions carried out by specific nerves innervating the airway. The nodose and jugular ganglia house sensory neurons that innervate the airway. The dashed line distinguishes central vs peripheral nervous system. It should be noted that the vagus nerve innervates many organs, including the GI tract and pancreas.*

mucociliary transport, and chronic airway infection and inflammation.[96] IV antibiotics in children with CF do not decrease cough frequency,[97] whereas they do in adults with CF.[98] Therefore, the mechanisms that mediate or the triggers that initiate cough may be different in children with CF vs adults with CF and not solely explained by infection. A different mechanism mediating cough is supported by the finding that children with CF have a decreased cough response to sensory nerve irritants compared with healthy and diseased control populations.[99] Of note, CFTR mRNA has been found in the nucleus of the solitary tract,[51,52] a brain region that regulates cough.[95,100] Thus, the neural mechanisms orchestrating cough may be different in CF.

Intestinal Obstruction and Motility

The GI tract is highly innervated. Recently, *CFTR* mRNA and protein have been found in the neuronal ganglia of the GI tract of humans.[101] Interestingly, the pattern and extent of neural innervation of the GI tract might be altered in some people with CF.[77,78] In Hirschsprung's disease, innervation of the intestine is absent, and intestinal blockage ensues.[102] These observations might suggest that some GI features of CF, such as meconium ileus,[103-105] intestinal obstruction, and constipation,[106] have a neural component.

Mucus

CF mice display defective submucosal gland secretion in response to sensory nerve stimulation,[107] whereas cholinergic stimulation reveals minor to moderate secretion defects.[104,108,109] Sensory nerves innervating the airway terminate in the nucleus of the solitary tract,[51,52] a key brain region that mediates submucosal gland stimulation and secretion.[110] Thus, it is possible that in CF, the neural circuits mediating submucosal gland secretion are abnormal.

Glucose Regulation

Cystic fibrosis-related diabetes (CFRD) is one of the most common comorbidities in people with CF.[111] CFRD is associated with more frequent pulmonary exacerbations,[112] more severe lung disease,[113] and greater mortality.[114] Prior to the development of overt diabetes, people with CF display a spectrum of glucose tolerance abnormalities.[115] Similar observations have been made in CF animal models.[76,116] Over time, insulin deficiency and insulin resistance develop. CFTR has been found in key neural regions, such as the hypothalamus and sympathetic nervous system,[56,58,61] that exert control over the allocation of glucose and endocrine pancreatic function.[117] In the hypothalamus, diminished CFTR activity impairs neuronal glucose-sensing properties.[65] Decreased neuronal glucose-sensing in specific subregions of the hypothalamus induces glucose intolerance and deficient insulin secretion.[118] Thus, it is possible that defects in the neural mechanisms governing endocrine pancreatic function and glucose regulation contribute to CFRD.

Conclusions

CF is a complex disease that affects many organs. Although the nervous system is not traditionally viewed as an organ affected in CF, limited evidence suggests that it is impacted and might contribute to CF pathogenesis. It is well accepted that chloride transport greatly modifies neuronal activity.[50] Thus it is predicted that the presence or absence of CFTR within the nervous system might influence neuronal function. Yet little is known regarding the role of CFTR in the nervous system or how the nervous system might contribute to CF. Further investigations that focus on neural CFTR in clinically relevant phenotypes, such as cough, GI obstruction, mucus abnormalities, and glucose regulation, are of considerable interest. The use of animal models with conditional CFTR expression might be of particular value in this regard. Alternatively, selective rescue of CFTR to distinct neural subcompartments might also yield significant information. Clinical assessment of the effects of CFTR potentiators and correctors on neural function in people with CF might also be highly interesting. Such studies might embrace noninvasive measurements of neural function, including auditory brainstem-evoked potentials, pupillary reflex, and aquagenic wrinkling. Carrying out any one of these lines of work might reveal new knowledge and unanticipated insight into CF pathogenesis.

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