be cleaved and activated by exogenous serine proteases such as trypsin, an action that is attenuated by the protease inhibitor aprotinin (Vallet *et al., Nature* 389:607 (1997)). When human bronchial epithelial cultures are mounted in Ussing chambers where native airway surface liquid is washed away, ENaC is predominantly active, suggesting that cell attached proteases are predominant (Bridges *et al., Am. J. Physiol. Lung Cell. Mol. Physiol.* 281:L16 (2001); Donaldson *et al., J. Biol. Chem.* 277:8338 (2002)). In contrast, under thin film conditions, where native airway surface liquid is present, ENaC activity is reduced, suggesting that airway surface liquid contains soluble protease inhibitors (Myerburg *et al., J. Biol. Chem.* 281:27942 (2006); Tarran *et al., J. Gen. Physiol.* 127:591 (2006); Gaillard et al., 2010 Pfleugers Arch, 460: 1-17).

[0004] Recently, it has been shown that the Short Palate Lung and Nasal epithelial Clone (SPLUNC1) protein comprises up to 10% of the total protein in the airway surface liquid and can readily be detected in both nasal lavage and tracheal secretions (Bingle, C. D., and Craven, C. J. (2002) PLUNC: a novel family of candidate host defense proteins expressed in the upper airways and nasopharynx *Hum Mol Genet* **11**, 937 ; Campos, M. A., et al. (2004) Purification and characterization of PLUNC from human tracheobronchial secretions *Am J Respir Cell Mol Biol* **30**, 184; Lindahl, M., Stahlbom, B., and Tagesson, C. (2001); Identification of a new potential airway irritation marker, palate lung nasal epithelial clone protein, in human nasal lavage fluid with two-dimensional electrophoresis and matrix-assisted laser desorption/ionization-time of flight *Electrophoresis* **22**, 1795). SPLUNC1 appears to be a volume sensing molecule since it can be secreted onto the mucosal surface of the superficial epithelia where ENaC is expressed (Bartlett *et al., J. Leukoc. Biol.* 83:1201 (2008); Bingle *et al., J. Pathol.* 205:491 (2005)). Furthermore, SPLUNC1 has been demonstrated to contain a subdomain that functions as an inhibitor of ENaC through its N-terminal domain.

[0005] The present invention discloses novel specialized non-naturally occurring peptides that mimic the properties of SPLUNC1 in regulation of sodium channels by binding to and inhibiting ion transport to regulate sodium absorption and fluid volume and treat disorders responsive to modulating sodium absorption.

SUMMARY

[0006] The present disclosure is based, in part, on the design of specialized non-naturally occurring peptides to regulate the activity of sodium channels. Accordingly, in one aspect, the invention refer to a fusion polypeptide comprising an epithelial sodium channel (ENaC) internalization-inducing peptide and a heterologous peptide, wherein

the ENaC internalization-inducing peptide is SEQ ID NO:127. In another aspect, the present invention refers to a pharmaceutical formulation comprising a fusion polypeptide as disclosed herein and a pharmaceutically acceptable carrier. In a further aspect, the present invention refers to use of a fusion polypeptide as disclosed herein in the manufacture of a medicament for treating a lung disorder, wherein the lung disorder is cystic fibrosis, non-cystic fibrosis bronchiectasis, chronic obstructive pulmonary disease, acute bronchitis, chronic bronchitis or asthma. In one example, there is disclosed a method of inhibiting the activation of a sodium channel, comprising contacting a sodium channel with a specialized non-naturally occurring peptide or a functional fragment thereof. In one example, the sodium channel is an epithelial sodium channel (ENaC). In one example, the specialized non-naturally occurring peptide or a functional fragment thereof binds to the sodium channel.

[0007] Another example describes a method of inhibiting sodium absorption through a sodium channel, comprising contacting the sodium channel with a specialized non-naturally occurring peptide or a functional fragment thereof. In one example, the specialized non-naturally occurring peptide or a functional fragment thereof binds to the sodium channel.

[0008] A further example describes a method of increasing the volume of fluid lining an epithelial mucosal surface, comprising contacting a sodium channel present on the epithelial mucosal surface with a specialized non-naturally occurring peptide or a functional fragment thereof. In one example, the specialized non-naturally occurring peptide or a functional fragment thereof binds to the sodium channel.

[0009] Another example describes a method of reducing the level of a sodium channel present on the surface of a cell, comprising contacting the sodium channel with a specialized non-naturally occurring peptide or a functional fragment thereof. In one example, the specialized non-naturally occurring peptide or a functional fragment thereof binds to the sodium channel.

[0010] A further example describes a method of treating a disorder responsive to inhibition of sodium absorption across an epithelial mucosal surface in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of a specialized non-naturally occurring peptide or a functional fragment thereof. In one example, the specialized non-naturally occurring peptide or a functional fragment thereof binds to the sodium channel.

[0011] Another examples describes a method of regulating salt balance, blood volume, and/or blood pressure in a subject in need thereof, comprising delivering to the subject a therapeutically effective amount of a specialized non-naturally occurring peptide or a functional fragment thereof. In one example, the specialized non-naturally occurring peptide or a functional fragment thereof binds to the sodium channel.

[0012] Another example describes a specialized non-naturally occurring peptide or a functional fragment thereof that mimics the sodium channel binding domain of a PLUNC protein and binds to a sodium channel, wherein cleavage of the sodium channel by a protease is inhibited when bound to the peptide.

[0013] Another example describes a kit comprising the peptide of the invention.

[0014] Another example describes the use of a specialized non-naturally occurring peptide or a functional fragment thereof for the preparation of a medicament to treat a disorder responsive to inhibition of sodium absorption in a subject in need thereof.

[0015] These and other aspects of the invention are set forth in more detail in the description of the invention below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Fig. 1 shows the sequence of S18 (SEQ ID NO:1). Highlighted in red are residues essential for ENaC interaction, while highlighted in blue are residues that were found not to contribute.

[0017] Figs. 2A-2B show the 1st half (LPVPLDQT (SEQ ID NO:113) but not the remainder (DQTLPLNVNP (SEQ ID NO:114) of S18 is required for inhibition of ENaC and preservation of ASL height.

[0018] Fig. 3A shows that S18 (SEQ ID NO:1) and aaLPVPLDQTLPLNVNPaa (SEQ ID NO:2) have equal potency and efficacy, despite SEQ ID NO:2 being flanked by D-alanines. Fig 3B shows that S18, and aaLPVPLDQTaa (SEQ ID NO:3) have equal potency and efficacy, despite (SEQ ID NO:3) being flanked by D-alanines. Fig. 3A also shows the relative potency of a sample of peptides, including aaLPNlePLDQTaa (SEQ ID NO:5), which displays increased potency, relative to S18 or SEQ ID NO:4.

[0019] Fig. 4 shows an additional comparison of S18 and SEQ ID NO:5, and increased potency of SEQ ID NO:5.

[0020] Figs. 5A-5U show the results of experiments analyzing the effects of various peptides on internalization of alpha-ENaC in HEK293T cells and effects on cell viability when GFP-tagged alpha-ENaC is co-expressed with beta and gamma ENaC. EC50 values are shown at the bottom of several of the figures. In these figures, ADG is a negative control peptide with the sequence: NH3-ADGGLLLLNNPPPPQTVV-NH2

CLAIMS

1. A fusion polypeptide comprising an epithelial sodium channel (ENaC) internalization-inducing peptide and a heterologous peptide, wherein the ENaC internalization-inducing peptide is SEQ ID NO:127.

2. The fusion polypeptide of claim 1, wherein a heterologous peptide is fused to the amino terminus of the ENaC internalization-inducing peptide or to the carboxy terminus of the ENaC internalization-inducing peptide.

3. The fusion polypeptide of claim 1, wherein a first heterologous peptide is fused to the amino terminus of the ENaC internalization-inducing peptide and a second heterologous peptide is fused to the carboxy terminus of the ENaC internalization-inducing peptide.

4. The fusion polypeptide of claim 3, wherein the first heterologous peptide and the second heterologous peptide are the same.

5. The fusion polypeptide of claim 1, wherein the heterologous peptide comprises one or more non-naturally occurring amino acids.

6. The fusion polypeptide of claim 1, wherein the heterologous peptide consists of one or more non-naturally occurring amino acids.

7. The fusion polypeptide of claim 1, wherein the heterologous peptide consists of two non-naturally occurring amino acids.

8. The fusion polypeptide of claim 1, wherein the heterologous peptide consists of D-Ala-D-Ala.

9. The fusion polypeptide of claim 1, wherein the fusion peptide consists of SEQ ID NO:128.

10. The fusion polypeptide of claim 1, wherein the fusion polypeptide induces cellular internalization of ENaC.

11. The fusion polypeptide of claim 1, wherein the fusion polypeptide induces cellular internalization of ENaC by HEK293T cells that express human alpha-, beta-, and gamma-ENaC.

12. A pharmaceutical formulation comprising a fusion polypeptide of claim 1 and a pharmaceutically acceptable carrier.

13. A pharmaceutical formulation comprising a fusion polypeptide of claim 9 and a pharmaceutically acceptable carrier.

14. The pharmaceutical formulation of claim 13, wherein the pharmaceutical formulation is to be administered to the lung as an aerosol.

15. Use of a fusion polypeptide of claim 1 in the manufacture of a medicament for treating a lung disorder, wherein the lung disorder is cystic fibrosis, non-cystic fibrosis bronchiectasis, chronic obstructive pulmonary disease, acute bronchitis, chronic bronchitis or asthma.

16. Use of a fusion polypeptide of claim 9 in the manufacture of a medicament for treating a lung disorder, wherein the lung disorder is cystic fibrosis, non-cystic fibrosis bronchiectasis, chronic obstructive pulmonary disease, acute bronchitis, chronic bronchitis or asthma.

17. The use of claim 16, wherein the fusion polypeptide is to be administered to the lung of the subject in need thereof.