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REVIEW

The Na_x (SCN7A) channel: an atypical regulator of tissue homeostasis and disease

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Abstract

Within an articulately characterized family of ion channels, the voltage-gated sodium channels, exists a black sheep, SCN7A (Na_x). Na_x, in contrast to members of its molecular family, has lost its voltage-gated character and instead rapidly evolved a new function as a concentration-dependent sensor of extracellular sodium ions and subsequent signal transducer. As it deviates fundamentally in function from the rest of its family, and since the bulk of the impressive body of literature elucidating the pathology and biochemistry of voltage-gated sodium channels has been performed in nervous tissue, reports of Na_x expression and function have been sparse. Here, we investigate available reports surrounding expression and potential roles for Na_x activity outside of nervous tissue. With these studies as justification, we propose that Na_x likely acts as an early sensor that detects loss of tissue homeostasis through the pathological accumulation of extracellular sodium and/or through endothelin signaling. Sensation of homeostatic aberration via Na_x then proceeds to induce pathological tissue phenotypes via promotion of pro-inflammatory and pro-fibrotic responses, induced through direct regulation of gene expression or through the generation of secondary signaling molecules, such as lactate, that can operate in an autocrine or paracrine fashion. We hope that our synthesis of much of the literature investigating this understudied protein will inspire more research into Na_x not simply as a biochemical oddity, but also as a potential pathophysiological regulator and therapeutic target.

Keywords SCN7A · Na_x · $Na_v2.1$ · Homeostasis · Disease

Introduction

Voltage-gated sodium channels

The voltage-gated sodium channel (VGSC) family consists of a family of heteromeric transmembrane proteins that regulate the flux of Na^+ ions through cell membranes to enable the production of electrical signals via generation and propagation of an action potential in excitable cells. Structural analyses indicate that VGSCs contain large, pore-forming α subunits and smaller, auxiliary β subunits. Mammalian genomes harbor genes to encode ten different α subunits, which display a high degree of sequence and structural homology albeit varied tissue expression patterns. Mammals also have genes that encode five different β subunits [1]. The α subunits contain the bulk of the structural material required for typical VGSC function including conduction of ions, voltage-sensing and voltage gating, inactivation, and transmembrane expression at the cell surface [2]. The β subunits are found to interact directly with the α subunits through disulfide or non-covalent bonding, depending on the β subunit in question. Early descriptions of β subunits described them as simple accessory proteins to their α counterparts, but further research has demonstrated critical roles of β subunits in more diverse functions including regulation of pharmacological channel properties, alteration of gating mechanisms and kinetics, and regulation of cellular

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excitability, even including some activities of β subunits that operate entirely independently of α subunits [2–4].

VGSC α subunits, encoded by the *SCN$_n$A* family of genes, are voltage-sensing and respond to activation via propagation of action potentials, with one exception. Na_x, sometimes referred to as NaG, Na_C, $Na_v2.1$, or $Na_v2.3$, is encoded by the gene *SCN7A*, sometimes referred to as *Scn6a* in rodents. This channel has lost its voltage-gated properties and instead has evolved a de novo function as a sensor of extracellular sodium. Review of much of the literature underlying the discovery of Na_x and the functions of Na_x within nervous tissues has been elegantly presented previously by Noda and Hiyama [5], but we will briefly summarize important paradigms here regarding Na_x signaling to motivate and provide context to our subsequent presentation of reports investigating Na_x expression and function in other tissues.

Evolution of a novel role by Na_x

Discovery and cloning of the genes encoding Na_x

Early study of the Na_x channel came from its cloning and identification, its tissue-specific expression, and its basic function in human and rodent tissues and cell lines. George et al. cloned the transcript, encoding a channel they designated $Na_v2.1$, from a human cardiac cDNA library. Authors also demonstrated transcript expression by Northern blot in human cardiac, skeletal muscle, and uterine tissues [6]. Felipe et al. cloned the transcript, which they called $Na_v2.3$, from a cDNA library constructed from mouse atrial tumor-derived cells. The authors also demonstrated expression of the transcript in mouse cardiac and uterine tissue, and demonstrated developmentally-regulated transcript expression in brain, cardiac, and skeletal muscle tissue [7]. Gautron et al. cloned the transcript, encoding a channel they designated "Na-G," from a rat astrocyte cDNA library. The authors demonstrated the expression of this transcript in brain astroglia and in Schwann cell cultures derived from the sciatic nerve and from dorsal root ganglion (DRG). The authors also described transcript expression in lung, cardiac muscle, and skeletal muscle [8]. Akopian et al. cloned the transcript, encoding a protein which they called "sodium channel-like protein (SCL-11)," from a rat DRG cDNA library. They then used Northern blot and PCR to identify expression of the transcript in cell lines and neonatal rat tissue, demonstrating positive expression in lung, pituitary, bladder, and vas deferens tissue, as well as in PC12 and C6 glioma cell lines. In situ hybridization demonstrated expression of transcript in myelinating Schwann cells and weak expression within neuronal cells. Ectopic expression of the gene in *Xenopus* oocytes failed to demonstrate voltage-gated channel activity [9]. Subsequent to multiple instances of cloning the transcript from multiple tissues derived from multiple organisms, it became clear that all of these transcripts, later renamed *SCN7A* in humans and *Scn7a* in rodents, encoded orthologues of the same protein, referred to here as Na_x.

Phylogenetic divergence of *SCN7A*

In humans, *SCN7A* is found within an SCNα gene cluster on chromosome 2, a cluster which includes *SCN1A*, *SCN2A*, *SCN3A*, *SCN7A*, and *SCN9A*. Of these genes, *SCN7A* appears to have arisen most recently. Phylogenetic analysis by Widmark et al. suggests that the *SCN7A* gene arose in a eutherian mammalian ancestor, due to the absence of this gene in opossum, platypus, lizard, and birds [10]. In addition to its relatively recent phylogenetic ancestry and rapid evolution generally, other evidence also points towards the evolution of a separate function for this gene. The regions of *SCN7A* that, in other VGSCs, are typically indispensable for proper voltage-gating function seem to be aberrant, since basic residues in domains III and IV are missing from the predicted protein sequences of Na_x at sites recognized to be critical for standard VGSC function in the sequences of all examined animals [6], and because the intracellular loop between domains III and IV contains a methionine to isoleucine substitution within a motif known to be critical for sodium channel inactivation [10, 11]. Thus, analyses of the primary structure of Na_x indicate a lack of typical VGSC function, in-line with its lack of such activity evaluated experimentally [9].

Na_x exhibits function atypical for a VGSC

A divergent, non-traditional role of *SCN7A* among sodium channels has not only been suspected due to structural analysis but has also been borne out of analyses examining its physiology. Initial findings that $Na_x^{-/-}$ mice demonstrated abnormal saline-intake behavior under conditions of water deprivation suggested that Na_x might function as a sensor of sodium ions [12]. Watanabe et al. described the expression and activity of Na_x in glial cells harvested from mouse subfornical organ (SFO), as these glial cells stained positive for Na_x and responded to increases in extracellular sodium concentration [13].

In addition to its expression and activity in glial cells, initial observations [12] of Na_x expression in neurons of some brain regions, including the amygdala, prompted further investigation into channel properties of Na_x expressed in excitable cells. Hiyama et al. describes the isolation of neurons from murine DRG and demonstrated that dissociated neurons from *Scn7a*$^{+/+}$ mice but not *Scn7a*$^{-/-}$ mice incubated in a high sodium solution demonstrated a tetrodotoxin-insensitive inward sodium current. To further confirm this finding, the authors rescued the knockdown by ectopic

expression of *Scn7a* in dissociated neurons isolated from *Scn7a*$^{-/-}$ mouse DRG, resulting in rescued response to high levels of extracellular sodium [14]. Further elucidation of the characteristics of Na_x proceeded via observation that mice lacking *Scn7a* lose the preference to fresh water over saline that is normally induced by experimental dehydration, and that this loss of preference could be reversed by ectopic expression of *Scn7a* in the SFO using an adenoviral expression vector, demonstrating a causal relationship between Na_x activity in the SFO and a behavior that depends on the sensation of sodium concentration [15]. Induction of Na_x expression in a neuroblastoma cell line (Neuro-2a) and subsequent patch-clamp analysis determined that channel responses to high levels of sodium, in the form of inward electrical currents, were quantitatively similar in neurons compared to glial cells, suggesting that this channel operates similarly in excitable and non-excitable cells of the nervous system. Ectopic expression of Na_x in neuro-2a cells also allowed for the identification of channel ion specificity. Na_x responded at comparable magnitudes to equimolar concentrations of sodium and lithium ions, with positive responses at decreased magnitudes detected for rubidium and cesium ions. Comparison of channel responses suggests that Na_x is a channel selective for monovalent ions of alkali metals, with decreased magnitude of reactivity to the larger ions of rubidium and cesium, though it is worth mentioning that the physiological relevance of Na_x activity in response to these other alkali metals is likely insignificant due to the implausibility of reaching such high concentrations of these ions in vivo [16]. A report by Tremblay et al. described the frequent expression of Na_x in neurons from the median preoptic nucleus (MnPO) of rats, but not of mice, and reasoned that rat Na_x is the sodium leak channel responsible for the sensation of Na^+ by MnPO neurons [17] This species-specific difference in Na_x expression was consistent with other observations, by the same group, of inter-species expression differences of Na_x between mouse and rat brain tissue [18]. Berret et al. also demonstrated that dissociated rat MnPO neurons that expressed Na_x also expressed Na^+/K^+-ATPase, and that Na_x co-localized at the subcellular level to a significant extent with the α1 subunit of Na^+/K^+-ATPase. Exposure of dissociated rat MnPO neurons to an artificial cerebrospinal fluid containing elevated concentrations of Na^+ evoked inward Na^+ current, which was partially inhibitable by administration of Na^+/K^+-ATPase inhibitors, demonstrating the dependence of Na_x-driven current on Na^+/K^+-ATPase activity [19]. This same group also demonstrated that MnPO neurons isolated from rats fed with high-salt diets demonstrated a greater degree of influence on Na_x permeability by Na^+/K^+-ATPase activity, compared to rats fed with normal diets or low salt diets, suggesting that environmental sodium levels modulate the interaction between Na_x and Na^+/K^+-ATPase. Culture of differentiated Neuro2a cells in vitro under conditions of high extracellular sodium for 48 h led to increased expression of both Na_x and the α1 isoform of Na^+/K^+-ATPase, as well as increased colocalization of these proteins. These in vitro findings further lend support to the idea that environmental changes in sodium levels, such as those induced by diet, regulate Na_x activity through modulating expression and co-localization of Na_x and the α1 isoform of Na^+/K^+-ATPase [20].

Glial Na_x channel activity enhances uptake of glucose and production of lactate as a signaling molecule

Evidence exists to suggest that Na_x serves as a signaling molecule, governing paracrine signaling from glial cells sensing high extracellular sodium, to effect responses in nearby neurons. Shimizu et al. demonstrated that, in rat glial cells, similar to what was described in rat MnPO neurons [19, 20], Na_x interacts directly with the Na^+/K^+-ATPase α1 subunit, resulting in sensitization of glial cells to Na^+-induced uptake of glucose. Overexpression of Na_x in rat glioma cells resulted in enhanced sodium uptake under conditions of high extracellular sodium, as well as a significant increase in glucose uptake that was mediated by the interaction of the C-terminal region of Na_x with Na^+/K^+-ATPase. SFO tissue of $Na_x^{+/+}$ mice cultured under conditions of high sodium demonstrated increased glucose uptake and lactate release, while similar cultures of SFO tissue isolated from $Na_x^{-/-}$ mice failed to demonstrate increased glucose uptake or lactate release in response to high sodium. The authors then demonstrated that this released lactate mediated signaling from glial cells to GABAergic neurons to control activity in the SFO, suggesting that Na_x^+ glial cells sense increases in sodium concentration to stimulate glucose metabolism and lactate release in order to modulate neuronal signaling in the SFO [21].

Na_x activity is regulated by endothelin signaling

Further investigation into the role of lactate production in glial cells has led to a better understanding of Na_x activity regulation. Though much remains to be elucidated about the underlying factors that regulate the expression and activity of *SCN7A*/Na_x, several reports have demonstrated critical roles of endothelin family signaling in the regulation of Na_x activity.

Unezaki et al. demonstrated that administration of lactate was sufficient for $Na_x^{-/-}$ mice to overcome functional recovery from sciatic nerve transection, in a manner dependent on monocarboxylate transporter activity. Primary murine Schwann cells cultured in the presence of exogenous endothelin 1 (ET-1) demonstrated increased release of lactate, in a manner dependent on ET_BR but not ET_AR

signaling, and this enhancing effect on lactate production induced by ET-1 was attenuated in Schwann cells derived from $Na_x^{-/-}$ mice [22]. Another report by Tu et al. demonstrated that lactate, as well as both ET-1 and ET-3, increased neurite outgrowth in neurons derived from DRG in a manner dependent on ET_BR and protein kinase C (PKC) activity [23]. Coupled with the demonstration that ET_BR was expressed in Schwann cells [23], these reports collectively suggest that ET/ET_BR signals via PKC to activate the complex of Na^+/K^+ ATPase and Na_x signaling in Schwann cells, regulating glucose uptake and metabolism, resulting in the release of lactate to mediate functional responses in nearby neurons.

More details underlying the relationship between endothelin signaling and Na_x activity were uncovered in a report by Hiyama et al. which demonstrated that, in glial cells of the SFO, Na_x tended to colocalize with ET-3 and ET_BR. Dehydrated mice also demonstrated robust upregulation of ET-3 expression in the SFO, a result that was replicated by incubation of SFO tissue in a high sodium solution, prompting further investigation of the relationship between ET-3 and Na_x signaling. Exogenous ET-3 was sufficient to induce sodium influx in an ET_BR-dependent manner in Na_x^+ cells dissociated from the SFO of $Na_x^{+/+}$ mice, while SFO cells dissociated from the brains of $Na_x^{-/-}$ mice did not respond to exogenous ET-3, demonstrating that the effects of ET-3 through ET_BR on sodium influx in these cells, cultured under standard concentrations of sodium, were mediated by active Na_x. Electrical currents induced by exogenous ET-3 or by an increase in extracellular sodium were demonstrated to be redundant, as increasing extracellular sodium of mouse SFO cells cultured in the presence of saturation levels of ET-3 increased current only minimally, suggesting that these signaling effectors (that is, high sodium ion concentration and ET-3) acted via the same pathway, presumably through induction of Na_x signaling. Further investigation revealed that agonism of ET_BR resulted in signaling through PKC/ERK, activation of which was necessary for induction of sodium influx, which was consistent with the demonstration that phosphorylation of ERK1/2 was markedly increased in the SFOs of dehydrated mice. Hiyama et al. also found that a subset of cells dissociated from SFOs of $Na_x^{+/+}$ mice demonstrated increased glucose uptake when stimulated with exogenous ET-3, and that the entirety of this subset of ET-3-sensitive cells stained positive for Na_x expression. Conversely, ET-3-responsive cells, as assessed by enhanced glucose uptake in response to ET-3 stimulation, were not detected in populations of cells dissociated from the SFOs of $Na_x^{-/-}$ mice. Similarly, lactate release from SFO tissues isolated from $Na_x^{+/+}$ mice but not from $Na_x^{-/-}$ mice was increased by treatment with exogenous ET-3, and was accompanied by increased spike frequency in neighboring GABAergic neurons, a finding not present in SFO tissue isolated from $Na_x^{-/-}$ mice [24]. Taken together, these data suggest that endothelin signaling is responsible for Na_x activation at physiological concentrations of sodium and that, in glial cells located in the SFO, Na_x activates MAPK signaling to promote glucose uptake and lactate production.

Recent reports have demonstrated additional mechanisms by which Na_x signaling in glial cells regulates neuronal activity. Two reports by Sakuta et al. [25, 26] further unravel a mechanism whereby increased sodium concentration in body fluids in response to dehydration leads to activation of Na_x in glial cells located in the organum vasculosum laminae terminalis (OVLT), leading to synthesis and release of epoxyeicosatrienoic acids (EETs). In turn, released EETs stimulate $TRPV4^+$ neurons in the OVLT to regulate water-intake behavior. In another report, Nomura et al. [27] describe a mechanism by which elevated sodium levels in bodily fluids are sensed by Na_x^+ glial cells in the OVLT, leading to not only release of lactate but also co-release of H^+. Exported H^+ ions, in turn, stimulate acid-sensing ion channel 1a (ASIC1A) in OVLT neurons projecting to the paraventricular nucleus (PVN), resulting in signaling through rostral ventrolateral medulla (RVLM) neurons, leading finally to the elevation of blood pressure.

The mechanism underlying the paradigm of Na_x signaling as a mediator of salt sensing and communication to neighboring neurons to effect physiological changes is simplified and summarized in Fig. 1.

Evidence for roles of Na_x in fibrotic and inflammatory pathologies

Na_x drives dermal fibrosis and inflammation

Recently, our lab has uncovered roles for Na_x in pathophysiological signaling in the skin. Previously, we demonstrated that transepidermal water loss (TEWL) induced by wounding leads to inflammation and subsequent tissue fibrosis. This inflammatory response is characterized by dehydration-induced increases in expression of numerous pro-inflammatory mediators including IL-1β, IL-8, COX-2, and TNF-α in the epidermis [28]. These changes in gene expression are attributed to increased sodium flux through the epithelial sodium channel (ENaC), and could be recapitulated in vitro both through increasing the concentration of sodium in the media used to culture keratinocyte monolayers, as well as through culturing stratified epithelium tissue in an incubator in the absence of ambient humidity, leading to a decrease in media volume and a subsequent increase in sodium concentration through media evaporation. In vitro supplementation of keratinocyte culture medium with extra sodium resulted in measurable sodium flux into keratinocytes, phosphorylation of Akt, and

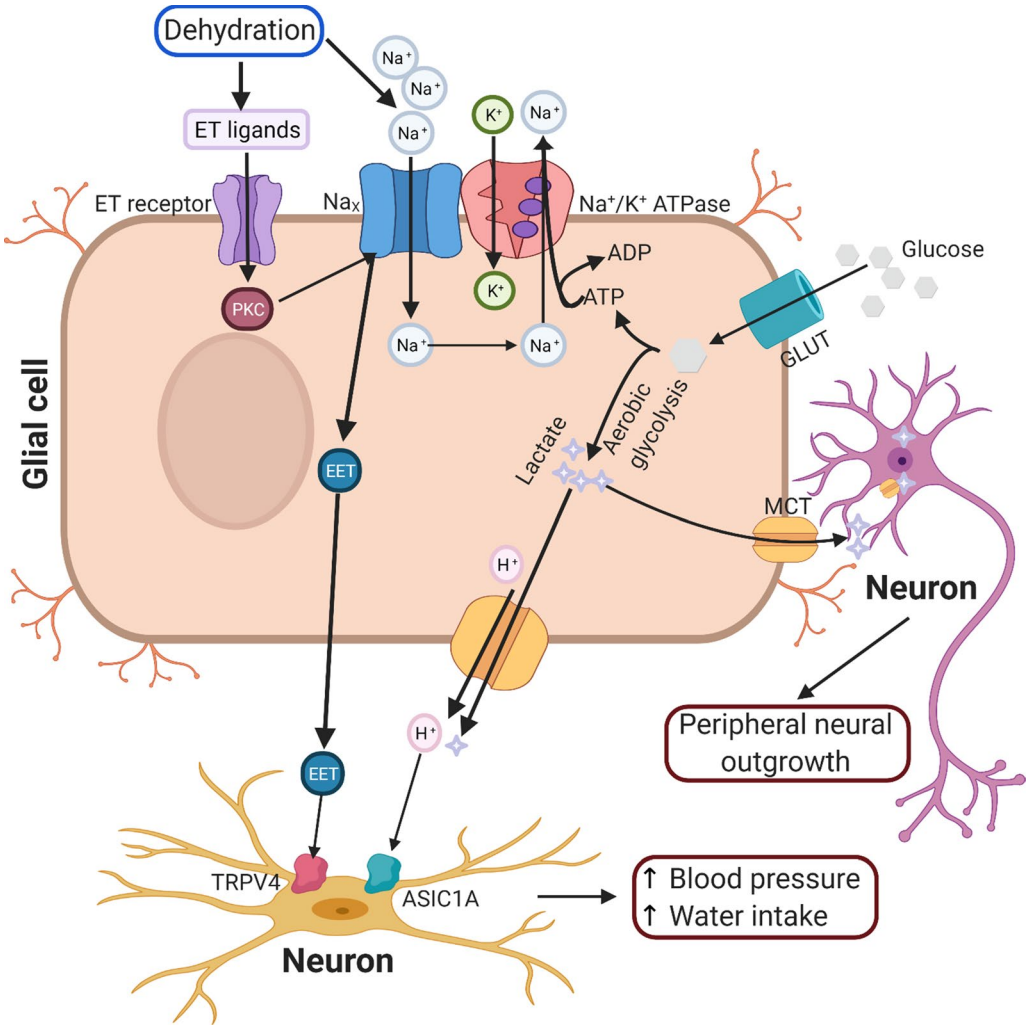

Fig. 1 Regulation of glial-neuronal crosstalk by Na_x signaling. Summary figure of neuronal regulation by glial Na_x signaling. Dehydration leads to local upregulation of endothelin (ET) ligands, which signal via their transmembrane receptors on glial cells in a manner dependent on protein kinase C (PKC), leading to subsequent sensitization of Na_x to concentrations of sodium that fall within the physiological range allowed within serum or cerebrospinal fluid. Alternatively, dehydration-induced increase in local sodium concentration activates Na_x, resulting in the production of current by inward sodium flux. Sodium import by Na_x and physical interaction with Na^+/K^+ ATPase enables functional coupling of these two channels, resulting in active export of Na^+ and consequent ATP hydrolysis. Energy depletion from extensive ATP hydrolysis results in enhanced glucose uptake via glucose transporters (GLUT), which is then metabolized via aerobic glycolysis to replenish ATP, producing lactate as a byproduct. Co-export of lactate with H^+ via a monocarboxylate transporter (MCT) enables lactate to modulate functional responses in neighboring neurons, leading to enhancement of peripheral neural outgrowth. Exported H^+ can activate the acid-sensing ion channel ASIC1A to promote elevation of blood pressure. Activation of Na_x can also lead to synthesis and release of epoxyeicosatrienoic acids (EETs), which can activate neuronal TRPV4, leading to regulation of water intake behavior

upregulation of *PTGS2*, the gene encoding COX-2, resulting in increased secretion of PGE_2. Keratinocytes cultured under conditions of reduced hydration were able to induce activation of co-cultured fibroblasts through soluble mediators, in a manner dependent on ENaC expression in the keratinocytes. In vivo pharmacological inhibition of ENaC or COX-2 in a rabbit ear hypertrophic scar model decreased scar formation, demonstrating that the induction of these pathways downstream of epidermal dehydration was sufficient to drive pro-fibrotic phenotypes in fibroblasts and subsequent development of tissue fibrosis [29].

We also demonstrated that ENaC expression overlaps significantly with an expression of Na_x across multiple epithelial tissues in mouse, including in the epidermis, suggesting a possible complementary role for these channels [30]. Since it had previously been described that Na_x is activated by increases in extracellular sodium ion concentration [14, 24], we suspected that the pro-inflammatory and pro-fibrotic

effects of TEWL-mediated reduced hydration in keratinocytes in vivo and in vitro might be regulated at least in part by Na_x. Indeed, we demonstrated that an increase in extracellular sodium was sufficient to induce inward sodium flux and activate Na_x, thereby activating ENaC via upregulation of the protease prostasin, leading to an increase in the expression of proinflammatory genes. Knockdown of *SCNN1A*, the gene encoding ENaC-α, or of *SCN7A*, was sufficient to antagonize the ability of keratinocytes cultured under reduced hydration conditions to induce fibroblast activation. Comparison of human hypertrophic scar tissue and human normal skin tissue revealed that Na_x is expressed at elevated levels in hypertrophic scar tissue. Additionally, in vivo knockdown of Na_x using siRNA was sufficient to reduce the formation of hypertrophic scar in a rabbit ear model, as well as to reduce the degree of dermal and epidermal thickening in a murine model of oxazolone-induced dermatitis, suggesting that this paradigm of keratinocyte-fibroblast communication induced by heightened local sodium concentrations is relevant to fibrosis and inflammatory pathologies in vivo as well [30].

Experimental simulation of dehydration via increasing the extracellular sodium concentration in keratinocyte cultures [30] was also demonstrated to induce expression of S100 family genes including those encoding S100A8, S100A9, and S100A12, low molecular weight calcium-binding proteins critical to the pathology of rheumatic and inflammatory skin diseases [31–33]. We then demonstrated that these proteins were present in aberrantly high quantities in keloid and hypertrophic scar tissue compared to healthy skin tissue. Dehydration induced epidermal expression of S100A8, S100A9, and S100A12, which was sufficient to induce fibroblast activation via RAGE-dependent and TLR4-dependent signaling. Administration of exogenous S100A8 and S100A12 resulted in greater deposition of hypertrophic scar tissue in a well-characterized rabbit ear model as determined by scar elevation index, lending greater credence to the hypothesis that these proteins pathologically induce the formation of hypertrophic scar under certain conditions [34, 35]. Recently, our lab has also demonstrated that Na_x is overexpressed in the skin of atopic dermatitis patient samples compared to control healthy skin. Further, knockdown of *SCN7A* using RNAi was sufficient to ameliorate inflammation and dermatitis symptoms, including aberrantly elevated expression of S100A9, in rabbit models of atopic dermatitis-like [36] and psoriasis-like [37] dermatitis. A summary of the therapeutic effects reported as a result of Na_x inhibition is presented in Table 1. Taken together, these reports suggest that activation of ENaC/Na_x signaling in the epidermis drives pro-fibrotic and pro-inflammatory processes through the propagation of dehydration-induced, pathological epidermal-dermal crosstalk.

Na_x expression is positively associated with renal fibrosis

Further evidence for Na_x as a potential regulator of tissue fibrosis comes from analyses of murine models of kidney injury and fibrosis. Proteomic analysis of a folic acid-induced murine model of acute kidney injury compared to vehicle control animals demonstrated upregulation of Na_x, suggesting that this sodium channel is upregulated in response to acute kidney injury [38]. In a more comprehensive analysis, Craciun et al. methodically sought to uncover peptide biomarkers for kidney fibrosis and utilized RNA-sequencing to discover genes that were induced over time in a folic acid-induced model of murine kidney fibrosis. One of the top ten candidate genes uncovered in this analysis was *Scn7a*. *Scn7a* transcript expression was induced in multiple murine kidney fibrosis models including those induced by intraperitoneal folic acid, unilateral ureteral obstruction (UUO), and unilateral ischemia-reperfusion injury. Na_x protein expression was demonstrated to be upregulated in a folic acid-induced mouse model of kidney fibrosis compared to a healthy mouse kidney, as well as in kidney tissue from humans with chronic kidney disease-induced fibrosis versus a healthy human kidney. Na_x expression scaled both positively and negatively in correlation with the severity of experimental kidney fibrosis, as would be expected if Na_x expression were causally involved with, or upregulated through compensation in, the pathology of kidney fibrosis. Expression of *Scn7a* was also upregulated in a murine model of liver fibrosis, though it was not found to be upregulated in fibrotic liver tissue from patients with primary sclerosing cholangitis. Since the authors of this report were interested in finding a translational biomarker specific to kidney fibrosis, and since *Scn7a* was demonstrated to be upregulated in a murine liver fibrosis model as well, this marker was not investigated further in this report [39]. The same group described increased expression of *Scn7a* transcript in a UUO model of murine kidney fibrosis [40]. Another report demonstrates that *Scn7a* expression was upregulated at 2 and 8 days post-operatively in a murine UUO model of kidney fibrosis, suggesting that this increase in expression is associated, seemingly reproducibly, with the development of kidney fibrosis [41]. Taken together, these data suggest that Na_x is upregulated in fibrotic kidney tissue and may contribute to pathologies associated with renal fibrosis and loss of renal function.

Na_x expression is dysregulated in other disease states

Other data supportive of a role for Na_x in disrupted homeostasis and in tissue fibrosis are sparse and correlative at best, often serving as overlooked footnotes or buried within large datasets, but we maintain that these data are worth

Table 1 Reports of therapeutic effects resultant from Na_x inhibition

Model	Treatment	Therapeutic effects	References
Rabbit ear excisional wound-induced hypertrophic scar	siRNA targeting *SCN7A* versus sham siRNA	Scar elevation index ↓ *PRSS8* ↓, *PTGS2* ↓, *IL1B* ↓, *CXCL8* ↓	[30]
Mouse oxazolone-induced dermatitis skin	siRNA targeting *Scn7a* versus sham siRNA	Epidermal thickening ↓ Dermal thickening ↓ *Prss8* ↓, *Ptgs2* ↓, *Il1b* ↓	
Rabbit ear imiquimod-induced dermatitis skin	siRNA targeting *SCN7A* versus sham siRNA	Erythema ↓ Excoriation ↓ Papulation ↓ Epidermal thickening ↓ Monocyte infiltration ↓ Epidermal hyperproliferation ↓ Loss of CK10 ↓ $CD3^+$ T cell infiltration ↓ Macrophage infiltration ↓ Neutrophil infiltration ↓ Mast cell infiltration ↓ Epidermal S100A9 ↓	[37]
Rabbit ear oxazalone-induced dermatitis skin	siRNA targeting *SCN7A* versus sham siRNA	Erythema ↓ Excoriation ↓ Papulation ↓ Epidermal thickening ↓ Monocyte infiltration ↓ Epidermal hyperproliferation ↓ Loss of CK10 ↓ Mast cell infiltration ↓ Eosinophil infiltration ↓ $CD3^+$ T cell infiltration ↓ Macrophage infiltration ↓ Epidermal TSLP ↓ Epidermal PAR2 ↓ Dermal *IL6* ↓ Epidermal *PTGS2* ↓ Epidermal *IL1B* ↓ Epidermal *CXCL8* ↓ Epidermal S100A9 ↓	[36]
Rabbit ear house dust mite extract-induced dermatitis skin	siRNA targeting *SCN7A* versus sham siRNA	Erythema ↓ Excoriation ↓ Papulation ↓ Epidermal thickening ↓ Monocyte infiltration ↓ Epidermal hyperproliferation ↓ Loss of CK10 ↓ Mast cell infiltration ↓ Eosinophil infiltration ↓ $CD3^+$ T cell infiltration ↓ Macrophage infiltration ↓ Epidermal TSLP ↓ Epidermal PAR2 ↓ Dermal *IL6* ↓ Dermal *IL4* ↓ Epidermal *PTGS2* ↓ Epidermal *IL1B* ↓ Epidermal *CXCL8* ↓ Epidermal S100A9 ↓	

mentioning, if only briefly, since Na_x is so poorly studied outside of nervous tissue. In hepatocytes isolated from rat livers regenerating after partial hepatectomy, expression of *Scn7a* was upregulated from 2 to 168 h post-hepatectomy suggesting that *Scn7a* upregulation may be a response mechanism to disrupted hepatic homeostasis as well [42]. Differentiation of a human hepatic stellate cell line with TGF-β1 resulted in significant upregulation of *SCN7A*

expression [43]. Transcriptomic analysis demonstrated that liver endothelial cells isolated from rats experiencing CCl_4-induced cirrhosis demonstrate increased expression of *Scn7a* compared to liver endothelial cells isolated from vehicle-treated rats [44]. In a report describing two murine models of cholestatic liver disease resulting in liver fibrosis (bile duct ligation or ingestion of 3,5-diethoxycarbonyl-1,4-dihydrocollidine), *Scn7a* was identified as a gene upregulated in both models [45]. As mentioned previously in reference to a report by Craciun et al., expression of *Scn7a* was upregulated in the liver tissue of mice fed α-naphthylisothiocyanate compared to control mouse liver, demonstrating that *Scn7a* is upregulated in a murine liver fibrosis model of a different etiology [39]. *Scn7a* expression was upregulated post-stroke in both young and aged rats, suggesting that *Scn7a* upregulation may be a consequence of tissue damage and/or loss of tissue homeostasis in the brain as well [46], consistent with Noda and Hiyama's description of unpublished data describing induction of Na_x expression in glial cells of damaged cortical tissue in response to traumatic brain injury [5]. Expression of human CFTRΔ508, the most common cystic fibrosis-causing mutation [47], in murine respiratory epithelium resulted in increased expression of *Scn7a* in lung tissue, possibly as a result of abnormalities in ion homeostasis [48]. Another transcriptomic analysis demonstrated that *SCN7A* transcript is upregulated in lung tissues of idiopathic pulmonary fibrosis patients compared to those of healthy control patients [49]. In a murine model of right ventricular remodeling resulting from intratracheal bleomycin-induced pulmonary hypertension, subcutaneous administration of a P2X7R antagonist dampened right ventricular maladaptive remodeling, fibrosis, and serum levels of IL-1β, which were associated with decreased expression of *Scn7a*, demonstrating that pharmacologic dampening of fibrosis is accompanied by decreased expression of *Scn7a* in this model [50]. Taken together, these data suggest that loss of tissue homeostasis may drive expression and activity of Na_x, which can serve as a response to the loss of tissue homeostasis and propagate signaling pathways that drive inflammation and tissue fibrosis. Reports of evidence describing the regulation of Na_x upon loss of tissue homeostasis are summarized in Table 2.

Modulation of Na_x signaling by endothelin and lactate

Endothelin signaling

As mentioned previously, endothelin signaling has been demonstrated to modulate Na_x activity both via ET-1/ET_BR [22] and ET-3/ET_BR [24] signaling, enabling activation of Na_x signaling even at homeostatic concentrations of tissue Na^+. While ET-3 expression is generally limited to the brain and pituitary gland, as well as lung and intestine, ET-1 is expressed generally by endothelial cells, and has been described in the kidney, nervous system, monocytes/macrophages and, under inflammatory conditions, in vascular smooth muscle cells and pulmonary epithelial cells as well. The endothelin peptides signal via two primary receptors in mammals, ET_AR and ET_BR. While ET_AR shows a strong preference for ET-1 and ET-2 versus ET-3, ET_BR shows a similar affinity for all three ligands [51]. Thus, it seems plausible to hypothesize that endothelin signaling may be responsible at least in part for the regulation of pathophysiological Na_x signaling outside of the scenarios outlined previously in nervous tissues.

Since we have previously demonstrated Na_x activation via dehydration [30], and since many inflammatory skin diseases are associated with disrupted barrier function and increased TEWL, it seems fair to hypothesize that Na_x might be activated through loss of epidermal barrier function and subsequent endothelin signaling in the skin, leading to the perpetuation of pro-inflammatory signaling. Accordingly, there have been numerous reports linking endothelin signaling to both inflammatory and fibrotic conditions. While an extensive review of this literature falls well outside of the scope of this manuscript, we believe it is worth highlighting some of these reports to support the hypothesis that pathological Na_x activity in inflammation and fibrosis might be related to endothelin signaling; if endothelin signaling is present and active in tissues and cell types that might express Na_x, and if endothelin has been demonstrated to induce pathological phenotypic changes in these cells similar to those in which Na_x has been implicated, then it is possible that Na_x signaling might be one paradigm by which endothelin signaling may mediate these pathophysiologies.

Pathological roles of endothelin signaling in fibrotic diseases

Endothelin signaling has been implicated in the pathology of multiple fibrotic diseases, since inhibition or ablation of endothelin signaling has been demonstrated to protect against fibrosis in a number of animal models mimicking fibrotic human pathologies of multiple organs, as summarized previously [52–55]. Here we highlight only a few reports out of many. Bleomycin-induced murine dermal fibrosis was ameliorated by treatment with dual endothelin receptor inhibitor bosentan [56], while ET_BR-knockout mice also resist bleomycin-induced scleroderma [57]. These findings are consistent with previously described reports demonstrating correlative and causative evidence that enhanced endothelin signaling is associated with sclerosis and scleroderma more specifically, including elevated ET-1 concentrations in the serum of patients with systemic sclerosis

Table 2 Reports of Na_x regulation upon loss of homeostasis

Model	Expression effect	Note	References
Differentiated murine neuroblastoma cells cultured in 155 mM [Na^+]	Cellular Na_x protein ↑ compared to cells cultured in 145 mM [Na^+]		[20]
Human hypertrophic scar skin	Epidermal Na_x protein ↑ compared to normal skin		[30]
Human atopic dermatitis skin	Epidermal Na_x protein ↑ compared to normal skin		[36]
Murine UUO-induced fibrotic kidney	Kidney *Scn7a* transcript ↑ compared to contralateral kidney		[39]
Murine I.P. folic acid-induced kidney fibrosis	Kidney *Scn7a* transcript/Na_x protein ↑ compared to vehicle-injected animal kidney	*Scn7a* transcript expression ↑ with increased folic acid dose, ↓ with therapeutic enalapril treatment	
Murine unilateral IRI-induced kidney fibrosis	Kidney *Scn7a* transcript ↑ compared to contralateral kidney		
Murine cisplatin-induced acute kidney injury	Kidney *Scn7a* transcript ↑ compared to vehicle-injected animal kidney		
Human fibrotic liver	Liver Na_x protein ↑ compared to healthy liver		
Murine ANIT-induced liver fibrosis	Liver *Scn7a* transcript ↑ compared to control liver		
Murine UUO-induced kidney fibrosis	Kidney *Scn7a* transcript ↑ compared to POD0 animal kidney		[40]
Murine UUO-induced kidney fibrosis	Kidney *Scn7a* transcript ↑ compared to sham animal kidney		[41]
Rat hepatectomy-induced liver regeneration	Isolated hepatocyte *Scn7a* transcript ↑ compared to healthy control		[42]
Rat CCl_4-induced liver fibrosis	Isolated hepatic endothelial cell *Scn7a* transcript ↑ compared to healthy control		[44]
Murine BDL-induced liver fibrosis	Liver *Scn7a* transcript ↑ compared to healthy control		[45]
Murine DDC-induced liver fibrosis	Liver *Scn7a* transcript ↑ compared to healthy control		
Rat carotid artery occlusion-induced stroke	Ipsilateral cortex *Scn7a* transcript ↑ compared to healthy control		[46]
Human traumatic brain injury	Damaged cortex glial cell Na_x ↑ compared to healthy tissue		[5]
Mice overexpressing $CFTR^{\Delta 508}$ in respiratory epithelium	Lung *Scn7a* transcript ↑ compared to healthy control lung		[48]
Human explanted IPF lungs	Lung *SCN7A* transcript ↑ compared to healthy control lung		[49]
Mouse bleomycin inhalation-induced pulmonary fibrosis	Lung *Scn7a* transcript ↑ compared to saline inhaled control lung ($P > 0.05$)	*Scn7a* transcript ↓ in PKT100-treated bleomycin mice versus vehicle-treated bleomycin mice ($P < 0.05$)	[50]
Mouse partial-thickness dermal abrasion	Skin *Scn7a* transcript ↑ compared to t = 0 skin		[133]
Human Brugada Syndrome patients	Endomyocardial tissue *SCN7A* transcript ↑ compared to healthy patients		[145]
Rat neonatal cardiomyocytes with siRNA-mediated knockdown of *Scn5a*	Cardiomyocyte *Scn7a* transcript ↑ compared to control vector-transduced cardiomyocytes		[146]

compared to those of healthy controls [57–60]. Autoantibodies for ET_AR were detected in the serum of patients with systemic sclerosis, and higher autoantibody levels were correlated with increased severity of systemic sclerosis disease manifestation and systemic sclerosis-related mortality [61]. Transgenic mice lacking expression of ET-1 in vascular endothelium were demonstrated to resist small pulmonary artery medial remodeling, arteriole muscularization, and pulmonary fibrosis resulting from inhalation of bleomycin as well [62]. In a murine model of pulmonary fibrosis, prophylactic administration of a selective ET_AR antagonist substantially increased survival and decreased

emergence of fibrotic lung pathology, including alterations in lung structure and mechanical properties, while dampening the inflammatory response and reducing collagen deposition [63]. Transgenic mice overexpressing ET-1 develop enhanced glomerulosclerosis, marked renal interstitial fibrosis, and accelerated declination of kidney function as they age, compared to age-matched wild-type controls [64]. Type II diabetic nephropathy rats displayed considerable renal fibrosis, associated with markedly increased renal ET-1 protein expression, and administration of an ET_AR antagonist resulted in partial protection against renal fibrosis [65]. Mice lacking expression of endothelin-converting enzyme 1, which is essential for ET-1 activation, resist pathological inflammation and kidney fibrosis induced by UUO [66]. More broadly, pharmacologic inhibition of endothelin has proven sufficient to blunt or reverse renal damage in a host of animal tissue models simulating various renal diseases [67]. Taken together, these reports demonstrate pathological roles for endothelin signaling in tissue fibrosis of various organs and suggest that endothelin inhibition may be a therapeutically useful anti-fibrotic intervention.

Association of endothelin signaling with inflammatory skin diseases

There also exists evidence of aberrant endothelin signaling in the pathology of inflammatory skin diseases, including atopic dermatitis. ET-1 was found to be dramatically upregulated in the epidermis of both murine skin in an atopic dermatitis model, as well as in human skin of atopic dermatitis patients, compared to healthy control skin of these same organisms [68, 69]. In a murine genetic model of atopic dermatitis with fibrotic characteristics resulting from skin-specific overexpression of IL-13, ET-1 was highly upregulated in the skin of mice upon continued inducible transgene expression of IL-13 compared to the skin of wild type mice. Treatment of these dermatitic mice with an antibody targeting thymic stromal lymphopoietin (TSLP) dampened the inflammatory response and markedly decreased ET-1 expression, suggesting that ET-1 expression scales with disease severity in this model of atopic dermatitis [70]. During the exacerbation of atopic dermatitis in human patients, plasma ET-1 levels were increased over those of healthy human subjects, and plasma ET-1 correlated positively with clinical severity, itch intensity, and serum IgE levels [71].

There also exists evidence for pathological roles of endothelin signaling in psoriasis. Compared to the plasma of healthy control patients, plasma of psoriatic patients is characterized by increased levels of general endothelin [72], ET-1 [73–76], and ET-2 [73]. Levels of ET-1 in both serum and lesional skin of psoriatic patients demonstrated statistically significant positive correlations with disease severity, as assessed by psoriasis area and severity index (PASI), and psoriatic lesional skin demonstrated increased expression of IL-8 compared to non-lesional skin, expression of which also correlated positively with ET-1 expression [76]. Nakahara et al. demonstrated aberrations in the epidermal expression pattern of ET-1 in psoriatic patients; ET-1 was highly expressed throughout the psoriatic epidermis, compared to the healthy epidermis in which ET-1 expression was limited to the stratum basale [69]. RNA-seq analysis of palmoplantar, scalp, and conventional plaque psoriasis patient tissues by Ingenuity Pathway Analysis denoted "ET-1 signaling" as the most enriched category of differentially expressed genes common to all of these manifestations, versus healthy control tissue, suggesting a generalized pathological role for endothelin signaling in psoriasis [77]. Pathological roles of endothelin signaling in atopic dermatitis and psoriasis, coupled with well-characterized impairments in epidermal barrier function in these diseases [78] and our observations that Na_x inhibition ameliorates symptoms in dermatitic models simulating these disease states [30, 36, 37], are consistent with the possibility that endothelin signaling may regulate Na_x activity to drive inflammatory skin diseases.

Endothelin signaling in epithelium

To assess the possibility that endothelin signaling might regulate Na_x activity in tissue epithelia, it is prudent to evaluate whether endothelin ligands and receptors are expressed in the epithelium and/or endothelium of tissues of interest that express Na_x, including the skin. Yohn et al. demonstrated the expression of ET-1 and ET_BR in neonatal human keratinocytes cultured in vitro [79]. Imokawa et al. also demonstrated the expression of ET-1 by human keratinocytes [80]. Expression of genes encoding ET-1, ET_AR, and ET_BR was upregulated in keratinocytes exposed to UVB radiation [81–83]. Treatment of HaCaT cells with ET-1 led to upregulation of IL-25, a cytokine critical to AD pathology, indicating active endothelin receptors in these cells [68]. Sah et al. also demonstrated that treatment of HaCaT cells with exogenous ET-1 induced proliferation and upregulated pro-angiogenic and pro-inflammatory gene expression [84]. Giaid et al. described the expression of high levels of ET-1 in airway epithelium and in type II alveolar epithelial cells in patients with cryptogenic fibrosing alveolitis compared to normal lung tissue [85]. Treatment of cultured human bronchial epithelial cells with TGF-β or TNF-α led to increased secretion of ET-1 via activation of NF-κB signaling [59]. Jain et al. described that alveolar epithelial cells produce ET-1 and express ET_AR and ET_BR, and that ET-1 drives epithelial-to-mesenchymal transition in alveolar epithelial cells via upregulation of TGF-β1 [86]. ET-1 also induced epithelial-to-mesenchymal transition in rat and human tubular epithelial cells [87, 88]. Treatment of human retinal pigment epithelial cells with exogenous ET-1 induced

proliferation, migration, and pro-fibrotic gene expression indicative of epithelial-to-mesenchymal transition [89]. Among others, these reports demonstrate that endothelin can be released, and that endothelin signaling pathways are active, in epithelial cells, suggesting that active endothelin signaling to regulate Na_x activity may be present in epithelial tissues in some scenarios.

Pathological roles of endothelin signaling in endothelium

As its name suggests, endothelin was identified as a vasoconstrictory peptide derived from endothelium [90], and thus much of the research of this signaling family has focused around endothelial roles of endothelin. Microvascular endothelial cell expression of ET-1 was increased in hypertrophic scar, keloid, and sclerotic skin tissue compared to healthy skin or mature scar tissue, suggesting a contribution of endothelin signaling in the endothelium to dermal fibrosis [91]. ET-1 cooperates with TGF-β to induce an endothelial-to-mesenchymal transition in murine pulmonary microvascular endothelial cells, potentially contributing to the formation of pulmonary myofibroblasts in pulmonary fibrosis [92]. Giaid et al. described increased endothelin-1 expression in endothelial cells of pulmonary arteries, preferentially those with medial thickening and intimal fibrosis, in patients with pulmonary hypertension, compared to control patients [93]. Patients with diabetic nephropathy and proteinuric IgA nephropathy displayed significantly increased ET-1 expression in glomerular and peritubular capillary endothelial cells compared to healthy controls, and ET-1 expression correlated significantly with proteinuria [94].

Pathological role for endothelin signaling in fibroblasts

Human lung fibroblasts were found to express both ET_AR and ET_BR, and ET-1 induced fibroblast proliferation and secretion of IL-6 [95]. In human lung fibroblasts, exogenous ET-1 also led to myofibroblast differentiation, pro-fibrotic gene expression paradigms, increased contractile activity, and conferred resistance to apoptotic stimuli [96–101]. Shi-Wen et al. also demonstrated that ET-1 secretion is increased substantially in sclerotic lung fibroblasts compared to healthy lung fibroblasts, and that sclerotic lung fibroblasts exhibit enhanced contractile activity [101]. In human dermal fibroblasts, ET-1 induced contraction and deposition of types I and III collagen [102]. Sclerotic human dermal fibroblasts treated with exogenous ET-1 demonstrated activated canonical TGF-β/SMAD signaling leading to myofibroblast differentiation [103]. In human myometrial fibroblasts, exogenous ET-1 drives myofibroblast differentiation [104]. Exogenous ET-1 dose-dependently induced expression of fibronectin and type III collagen in aged rat cardiac fibroblasts [105]. In human colonic subepithelial myofibroblasts, ET-1 induced a rapid calcium influx and dose-dependently induced increases in contractile force [106]. In primary human colon fibroblasts, ET-1 induced migration and contractile activity, while upregulating numerous pro-inflammatory and pro-fibrotic genes [107]. Guarda et al. demonstrated that both ET-1 and ET-3 dose-dependently induced collagen synthesis in rat cardiac fibroblasts [108], while Peacock et al. reported that ET-1 and ET-3 dose-dependently induced proliferation and chemotaxis in rat pulmonary arterial fibroblasts [109]. Together, these numerous reports demonstrate that fibroblasts isolated from multiple organs are capable of functional endothelin signaling, suggesting that endothelin signaling could potentially regulate Na_x activity, if Na_x is present and otherwise functional in fibroblasts.

Pathological roles of lactate in dermatitis and fibrosis

As described above, activation of Na_x in glial cells by high sodium leads to increased glucose uptake and increased production of lactate, due to functional coupling of Na_x with Na^+/K^+-ATPase, leading to functional effects regulated by lactate as a signaling molecule [21]. If this functional coupling persists in other cells and tissues expressing Na_x, including subsequent modulation of glucose uptake and lactate production, then it is possible that Na_x activity-induced production of lactate is relevant to other pathological tissue states as well. The α1 isoform of Na^+/K^+-ATPase is ubiquitously expressed across tissues [110], including those demonstrated to express Na_x, further supporting this possibility. If $Na_x/Na^+/K^+$-ATPase signaling outside of nervous tissue is pathologically induced and leads to induction of lactate production and release, this could serve as a potential mechanism through which Na_x activity regulates inflammatory and fibrotic pathologies.

Several studies point towards a potential role for lactate in dermatitis. In adult patients with refractory atopic dermatitis, serum LDH positively correlated with eczema area and severity index (EASI) scores [111]. Morishima et al. describe a correlation between serum LDH activity and cutaneous symptoms in children with atopic dermatitis, as well as increased LDH activity in epidermal tissue of children with severe atopic dermatitis compared to control skin [112]. Mukai et al. describe elevated levels of serum LDH activity in atopic dermatitis patients that scaled positively with symptomatic exacerbation and scaled negatively with symptomatic alleviation [113]. In patients with moderate to severe atopic dermatitis, baseline serum LDH levels predicted poor therapeutic response to dupilumab [114].

Numerous analyses point towards a critical role for lactate in tissue fibrosis. Analyses of lung homogenates from

patients with IPF demonstrate increases in lactate content compared to healthy lungs [115–117], as do renal homogenates from rat UUO kidneys versus those from control kidneys [118], and lung homogenates and BALF from murine bleomycin-instilled lungs versus control lungs [117, 119]. Keloid fibroblasts demonstrate enhanced rates of glycolysis compared to normal fibroblasts [120], consistent with reported roles for lactate in promotion of the synthesis and maturation of collagen [121]. Kottmann et al. also demonstrated increased lactate concentration in homogenates of IPF lungs versus healthy lungs, and analyzed this relationship in far more detail. Authors first described that treatment of primary human lung fibroblasts with TGF-β led to increased production of lactate and subsequent media acidification, and that exogenous lactate treatment-induced myofibroblast differentiation. The authors then described that, in IPF lung tissue, lactate dehydrogenase 5 (LDH5) was upregulated not only in myofibroblasts within fibroblastic foci but also in epithelial cells adjacent to fibroblastic foci, suggesting a role for paracrine lactate signaling in fibroblast activation in IPF [122]. Proof of concept experiments in lung fibroblasts have demonstrated the potential for lactate dehydrogenase inhibition to antagonize myofibroblast differentiation in vitro and in vivo [123–126], suggesting further relevance of this strategy for the treatment of tissue fibrosis.

Several studies also implicate the role of Na^+/K^+-ATPase in tissue fibrosis more generally. Recent reports demonstrate the potential of Na^+/K^+-ATPase inhibition to antagonize TGF-β signaling and myofibroblast differentiation in human fibroblasts cultured in vitro [127–129], and further reports demonstrate potential for Na + /K + -ATPase inhibition to ameliorate pathological symptoms in fibrotic diseases in vivo as well [130–132]. Taken together, alongside the paradigm of Na_x activity coupling with glucose uptake and lactate production via Na^+/K^+-ATPase, these data suggest other mechanisms through which Na_x signaling may potentially regulate tissue fibrosis.

A summary of some potential mechanisms through which Na_x signaling might drive pathophysiological tissue phenotypes based upon the above discussion is presented in Fig. 2.

Other potential roles of Na_x

Effects of Na_x in wound healing

In a mouse model of partial-thickness dermal abrasion characterized by extensive RNA-sequencing and analysis, *Scn7a* was upregulated 36–192 h post-wounding, which corresponds with wound re-epithelialization, suggesting that *Scn7a* upregulation may be involved in the re-epithelialization process in wound healing of the skin [133]. Since we had previously demonstrated functional roles for epidermal Na_x, we recently generated a global knockout mouse lacking expression of functional Na_x in order to better understand the role of Na_x in the healing of excisional wounds [134]. We utilized a splinted dorsal excisional wound model [135] in these mice to minimize wound contraction and force wound healing to proceed via granulation and epidermal migration, in a manner more closely representative of wound healing in human skin, and we characterized wound healing in $Na_x^{-/-}$ mice compared to $Na_x^{+/+}$ mice. From this analysis, we demonstrated that mice lacking functional Na_x displayed a decreased rate of wound healing due to delayed epidermal migration and decreased keratinocyte proliferation, consistent with our previous findings that knockdown of Na_x in HaCaT cells inhibited migration and differentiation [30]. Interestingly, $Na_x^{-/-}$ mice failed to demonstrate protection against inflammation induced by imiquimod or oxazolone, in contrast to our previous reports demonstrating protective effects of Na_x inhibition in inflammatory skin models [30, 36, 37], and wound epidermises in Na_x-/- mice demonstrated increased expression of some inflammatory genes compared to wild type mice during the healing process, suggesting that mice lacking Na_x maintain responsiveness to inflammatory stimuli. Though it is not clear the mechanisms by which $Na_x^{-/-}$ mice maintain responsiveness to inflammatory stimuli, their overall health and viability lends further reason to believe that these mice might be undergoing some sort of compensation. Though the mechanisms underlying functional compensation are not yet clear, some overlap exists between the signaling pathways activated by changes in osmolality and those activated by elevations in extracellular sodium [30], suggesting that osmosensory mechanisms might contribute to these phenomena. Additionally, Sakuta et al. have recently demonstrated that SLC9A4 expressed in the OVLT serves as a sensor of elevations in extracellular sodium concentration [136]. This demonstrates that the function of Na_x as an extracellular sodium sensor, while atypical as a member of the *SCN$_n$A* class of genes, is not entirely unique among all mammalian proteins. Future research will seek to understand why compensatory mechanisms potentially present in the skin of $Na_x^{-/-}$ mice that enable response to inflammatory stimuli do not also lead to a recapitulation of water preference over saline upon dehydration, as described previously in $Na_x^{-/-}$ mice [12, 15].

Expression of Na_x in lung tissue

As previously mentioned, early studies of *SCN7A* transcript focused on expression in cell lines and in whole tissues via RT-PCR and Northern blot. Recently, more in-depth analyses, as well as the wealth of data available from high-throughput technologies like single-cell RNA-seq, have enabled deeper analyses into the expression of this gene and others.

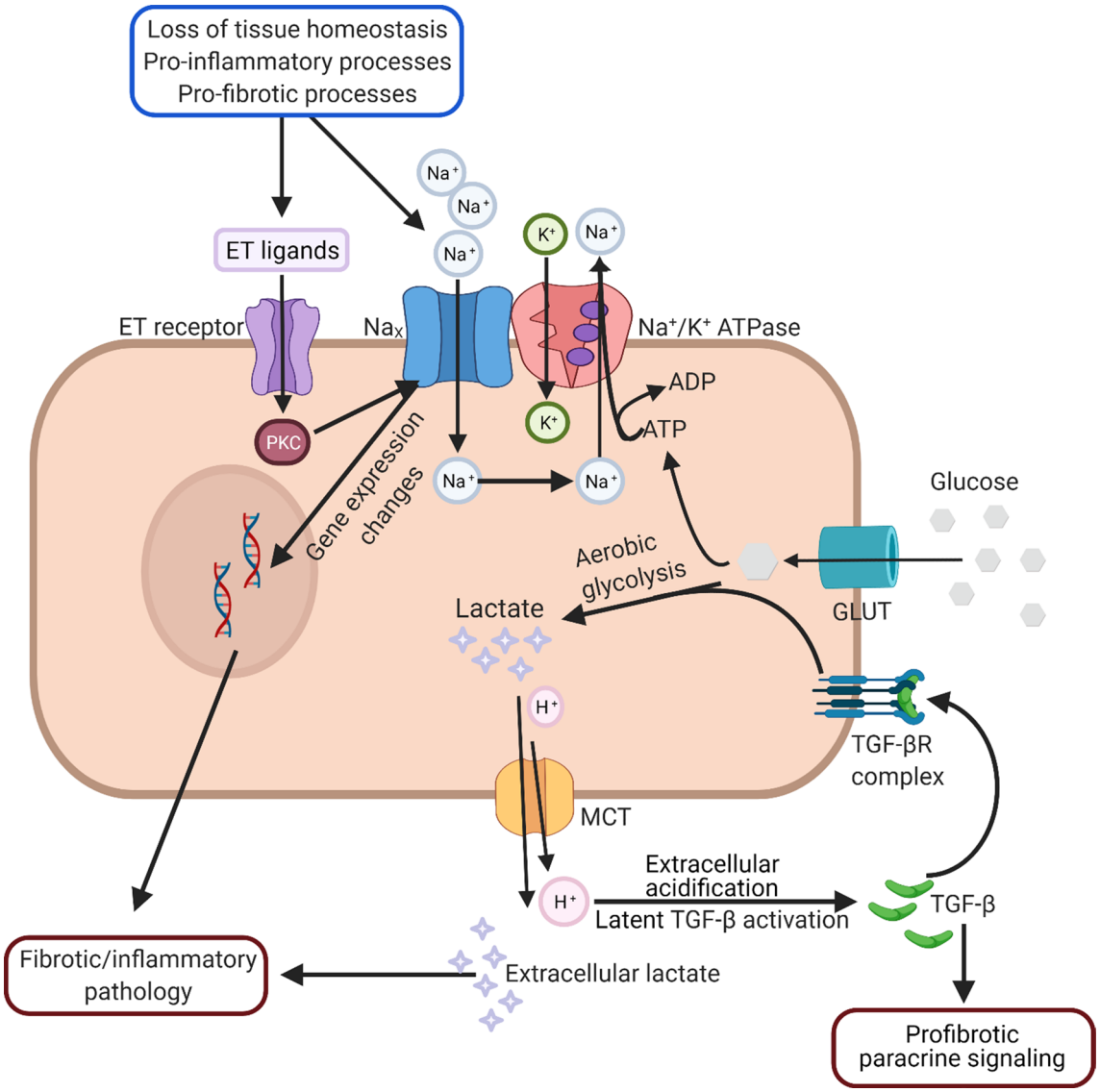

Fig. 2 Potential mechanism for Na_x signaling response to the loss of homeostasis. Simplified proposed figure for Na_x signaling as a response to the loss of tissue homeostasis. Tissue damage, pro-inflammatory processes, or pro-fibrotic processes may lead to activation of Na_x signaling in Na_x^+ cells via local hypernatremia and/or activation of ET receptor-mediated signaling on these cells via local secretion of ET ligands. Activation of Na_x signaling may lead directly to signal transduction pathways that result in upregulation of genes causally implicated in pathophysiological inflammation or tissue fibrosis. Additionally, functional coupling of Na_x with Na^+/K^+ ATPase may result in enhanced glucose uptake into Na_x^+ cells and subsequent aerobic glycolysis, resulting in the production of lactate. Lactate can be exported from the cell via MCT proteins, where it can contribute to inflammatory or fibrotic pathologies via novel or previously characterized mechanisms. In parallel, co-export of H^+ ions with lactate via MCT may lead to local extracellular acidification, which can activate latent TGF-β, leading to autocrine or paracrine signaling that drives fibrosis. Signaling via TGF-β can also drive cellular metabolic changes that promote aerobic glycolysis, thus increasing the production of lactate and furthering the cycle of pathological lactate signaling

As early reports of *SCN7A* described high levels of expression in lung tissue homogenate, more recent reports have enabled investigation of this phenomenon in more depth. Platoshyn et al. described expression of detectable *SCN7A* transcript in cultured human pulmonary artery smooth muscle cells [137]. Hagiwara et al. demonstrated expression of *Scn7a* transcript and Na_x protein in murine alveolar wall but not in the bronchial epithelium. Immunoelectron microscopy of murine lung tissues demonstrated the presence of Na_x protein in the basolateral membrane of pulmonary microvascular endothelial cells (PMVECs), as well as in the apical membrane and the cytoplasm of type II alveolar epithelial cells. PMVECs isolated from mouse lung expressed Na_x protein and *Scn7a* transcript, lending further

validity to the authors' findings denoting expression in endothelial cells in vivo. Exposing lung slices to an increase in sodium concentration in the presence of the ENaC blocker amiloride resulted in increased influx of Na^+ ions into the alveolar wall, suggesting that Na_x is functional in this tissue [138]. A seminal report by Reyfman et al. utilized single-cell RNA-seq to analyze > 76,000 cells total from 8 healthy donor lung biopsies and 8 lung explants from human patients with pulmonary fibrosis. Further analysis of this data reveals positive expression of *SCN7A* in some human pulmonary fibroblasts, mostly those obtained from healthy donor lungs, as well as weak positive expression in a small proportion of mast cells. Interestingly, a similar analysis of > 13,000 cells isolated from two healthy mouse lungs demonstrated positive expression of *Scn7a* not only in fibroblasts but also in endothelial cells, including alveolar capillaries, indicating potential differences in cell type-specific expression of Na_x among different species [139]. Sabbagh et al. performed RNA-seq analysis on endothelial cells isolated from murine brain, liver, lung, and kidney harvested on post-natal day 7 in *Tie2-GFP* mice, which express GFP specifically in endothelial cells. Differential gene expression in endothelial cells between tissues was used to uncover tissue-specific endothelial cell markers, and authors identified *Scn7a* as an endothelial marker specific to endothelial cells of pulmonary origin [140], though Barry et al. have demonstrated expression of *Scn7a* in glomerular capillaries as well [141]. In contrast, Watanabe et al. described pulmonary expression of Na_x in mice as occurring in alveolar type II cells [142]. Conflicting information regarding the cell types that express and do not express Na_x should prompt further research into tissue-specific and cell-type-specific expression of this protein; all reports of which we are aware demonstrate significant expression of Na_x in lung tissue, generally, even as the cell types purported to express it vary.

Expression of Na_x in heart tissue

Expression of transcript encoding Na_x was first described in cardiac tissue by George et al. who demonstrated expression, by Northern blot, in tissue lysates of human cardiomyopathic right atrium, cardiomyopathic left ventricle, and normal left ventricle [6]. Fifteen years later, Gaborit et al. described abundant expression of *SCN7A* transcript in healthy human right atrial, right ventricular, and Purkinje fiber tissues, second in quantity only to *SCN5A* among the VGSC family [143]. Previous analyses had demonstrated that individuals with Brugada Syndrome (BS), the inheritance of which carries increased risk of life-threatening ventricular arrhythmia, frequently harbored mutations in the *SCN5A* gene, resulting in alterations to the sequence of its encoded sodium channel, $Na_v1.5$ [144]. Thus, Gaborit et al. studied a series of patients with BS to determine aberrations in the expression of ion channels within their cardiac tissue. The authors determined that expression of *SCN7A* was significantly upregulated in endomyocardial tissues derived from BS patients compared to those of healthy patients, as well as from those of patients with arrhythmogenic right-ventricular cardiomyopathy or right-ventricular outflow-tract tachycardia, suggesting that the phenomenon of *SCN7A* upregulation was specific to BS among these conditions. The authors' interpretation of this finding was that upregulation of *SCN7A* in BS patients might serve as a compensatory mechanism for the downregulation of *SCN5A* or loss-of-function of $Na_v1.5$ frequently observed in these patients [145]. Recently, our lab aimed to further investigate this possibility. We demonstrated that knockdown of *Scn5a* in rat neonatal cardiomyocytes resulted in consequent upregulation of *Scn7a*, lending support to the previous relationship proposed by Gaborit et al., while knockdown of *Scn7a* led to downregulation of *Scn5a* [146]. $Scn5a^{+/-}$ mice, which expressed reduced amounts of functional $Na_v1.5$ and thus likely express increased Na_x, have also been observed to develop substantial cardiac fibrosis alongside their measured aberrant electrocardiographical parameters [147, 148]. Though this fibrosis is likely due in large part to lack of functional $Na_v1.5$, which is critical to the proper functioning of cardiac tissue, it is also a possibility that elevated Na_x expression is a pathophysiological contributor to the cardiac fibrosis exhibited by these animals, particularly since cardiac tissue exhibiting dampened expression of its primary sodium channel might be expected to have aberrant levels of extracellular sodium, potentially leading to increased Na_x activation as well, though this potential mechanism is entirely speculative. Taken together, these data suggest that the relatively high level of Na_x expressed in healthy cardiac tissue is indicative of the existence of functions of this sodium channel in the heart, potentially related to regulation of $Na_v1.5$ and/or excitation–contraction coupling, and that aberrantly high expression of *SCN7A*, such as that demonstrated by cardiac tissue of patients with *SCN5A* mutations or Brugada Syndrome, may contribute to cardiac pathology. There also exist data in conflict with the idea that the presence of cardiac Na_x is solely or predominantly a result of the expression in cardiomyocytes. A recent report described the expression of Na_x in rat cardiac fibroblasts but not in rat cardiomyocytes [149], suggesting that cardiac *fibroblasts* contribute the main source of Na_x detected in cardiac tissue. In support of this finding, self-assembling cardiac microtissues composed of a combination of mesenchymal stem cells and embryonic stem cell-derived cardiomyocytes demonstrated significant upregulation of *SCN7A* and gained the ability to respond to TGF-β, compared to cardiac microtissues composed solely of embryonic stem cell-derived cardiomyocytes [150], suggesting mesenchymal origin of *SCN7A* expression. Very recently, Simon-Chica et al. also described the expression of *Scn7a* in murine cardiac macrophages [151]. Seemingly

contradictory conclusions inspired by these reports further the case that the expression and role of cardiac Na_x, like pulmonary Na_x, merits further study to resolve these discrepancies and better understand the physiological and pathophysiological roles of Na_x in these tissues.

Conclusion

Examination of much of the literature describing the expression and activity of the atypical sodium channel Na_x, as we have performed here, answers several questions but raises far more. Translation of basic concepts underlying Na_x biochemistry and physiology from the nervous tissues in which they were uncovered to other tissues in which Na_x is expressed has revealed some key information underlying roles of Na_x in other tissues, but the consequences of physiological and pathophysiological sensation of $[Na^+]$ by Na_x must be subjected to far more study before a clearer picture can be uncovered. Further study of Na_x is of interest not just for neuroscientists, who would be expected to benefit, and in many cases already have benefited, from a greater understanding of Na_x, but also to researchers of many other disciplines. Our demonstration of ameliorative effects of acute Na_x inhibition in dermatitic models [30, 36, 37] suggests potential fruitful yield from study of Na_x and regulation of its activity for the purposes of inflammation and dermatology. The clear induction of Na_x expression in kidney injury and fibrotic renal tissue [38–40] merits further study of Na_x in renal physiology and pathology. Literature left undiscussed here describes statistically significant associations of *SCN7A* expression with tumor staging, *TP53* mutation status, and disease prognosis in cases of lung squamous carcinoma, non-small cell lung cancer, breast cancer, and esophageal squamous cell carcinoma [152–155], just to name a few, suggesting a potential role for this gene as an oncologic biomarker. Analysis of a Han Chinese population revealed novel SNPs in the *SCN7A* gene, one of which was significantly associated with essential hypertension [156], and a larger follow-up study also found a significant correlation between the presence of *SCN7A* SNPs and risk of hypertension [157]. As large sets of genomic, transcriptomic, and proteomic data from human patients and pre-clinical disease models are analyzed and made available, and as expression signatures continue to be generated and robustly verified in all cell types in the human body, we will no doubt come to a better understanding of the atypical sodium channel Na_x and any therapeutic avenues that may open up from revelation of its secrets. Further research should aim to better understand tissue-specific and organ-specific mechanisms through which Na_x acts in pathological scenarios, in order to evaluate the potential of Na_x inhibition as a therapeutic strategy for a variety of tissue pathologies. If promising, this research may further the argument for prioritization of discovery of Na_x inhibitors, including small molecules, which can then be validated in vitro and in preclinical models, with the hopes of future clinical promise.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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