ENaCs and ASICs as therapeutic targets

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Qadri YJ, Rooj AK, Fuller CM. ENaCs and ASICs as therapeutic targets. Am J Physiol Cell Physiol 302: C943–C965, 2012. First published January 25, 2012; doi:10.1152/ajpcell.00019.2012.—The epithelial Na⁺ channel (ENaC) and acid-sensitive ion channel (ASIC) branches of the ENaC/degenerin superfamily of cation channels have drawn increasing attention as potential therapeutic targets in a variety of diseases and conditions. Originally thought to be solely expressed in fluid absorptive epithelia and in neurons, it has become apparent that members of this family exhibit nearly ubiquitous expression. Therapeutic opportunities range from hypertension, due to the role of ENaC in maintaining whole body salt and water homeostasis, to anxiety disorders and pain associated with ASIC activity. As a physiologist intrigued by the fundamental mechanics of salt and water transport, it was natural that Dale Benos, to whom this series of reviews is dedicated, should have been at the forefront of research into the amiloride-sensitive sodium channel. The cloning of ENaC and subsequently the ASIC channels has revealed a far wider role for this channel family than was previously imagined. In this review, we will discuss the known and potential roles of ENaC and ASIC subunits in the wide variety of pathologies in which these channels have been implicated. Some of these, such as the role of ENaC in Liddle’s syndrome are well established, others less so; however, all are related in that the fundamental defect is due to inappropriate channel activity.

hypoactivity; cystic fibrosis; pain; epithelia; central nervous system; peripheral nervous system; cancer; acute respiratory distress syndrome; neurodegenerative disease; epithelial sodium channel; acid-sensitive ion channel; degenerin

THE ROLE OF ANY ION CHANNEL in a disease process can often be distilled down into inappropriately increased or decreased activity. This translates to a problem in one of two parameters; either too many or too few channels are expressed at the cell surface (N), or the channel is open for too long or not long enough (open probability or Popen). The macroscopic current (I) that is passed by a population of like channels in a cell is the product of these two numbers and the single-channel current, i(N = iPopen). Typical determinants of N include pathways that regulate trafficking of channels to and from the cell surface, the number of channels expressed in the cell, and the number of active (as opposed to silent or cryptic) channels, at the surface. Determinants of Popen include posttranslational modifications, e.g., phosphorylation, mutations, or the presence of pharmacological or endogenous agonists and blockers. In some cases, a single modification can affect both N and Popen, e.g., a mutation or interactions with the cytoskeleton. Multiple pathological conditions have now been associated with epithelial Na⁺ channels (ENaCs) and acid-sensitive ion channels (ASICs), although not all are due to channel hyper/hypo activity, but are simply the physiological response to a change in the environment, e.g., opening of ASIC channels in response to the fall in tissue pH mediating the pain caused by ischemia. Functional expression of ENaCs is also regulated by a large number of ancillary proteins such as serum and glucocorticoid kinase-1 (SGK1), a pluripotent enzyme that influences ENaC transcription and internalization (44, 238), and vasopressin, which decreases cellular retrieval (363). Several of these mechanisms have been covered in detail in a number of excellent recent reviews (59, 114, 115, 135, 164, 281, 341, 360), and only a brief outline of these different regulatory systems will be given here. In this review, we will focus on the pathologies in which ENaCs and ASICs have been directly implicated, and examine the potential for ENaC and ASIC-targeted therapeutics (see Table 1).

Conditions Associated With ENaC Hyperactivity: Hypertension and Cystic Fibrosis

Widely regarded as a milestone in renal physiology, the cloning of ENaC by Bernard Rossier’s group in the early 1990s (64, 66) has had a profound impact on cell physiology as a whole. ENaC is now widely accepted as a trimeric heteromer composed of α-, β-, and γ-subunits (151, 204, 362), and there are few tissues that do not express subunits of ENaC or one of its relations in the degenerin (Deg)/ENaC superfamily (see Fig. 1 and Table 2). Although originally expression cloned from the colon of salt-deprived rats, the significance of these studies lay in the assignment of a molecular identity to the Na⁺ channel of the renal collecting duct. In this location, the channel is involved in Na⁺ reabsorption from the tubular fluid. Although a majority (∼90–95%) of the daily filtered sodium load is reabsorbed in the proximal tubule and the thick ascending limb...
of the loop of Henle, it is ENaC that is chiefly responsible for the fine tuning of the final urinary Na+/H+ excretion. Inappropriate elevations in this small amount of Na+/H+ reabsorption via ENaC can lead to the development of hypertension, a major cause of mortality and morbidity in the industrialized world, with 31.3% of adults being affected in the US alone (72a).

The most common form of hypertension, essential hypertension, is a classic multifactorial disease, with both environmental and genetic factors. To date, the products of over 150 separate genes are thought to influence blood pressure regulation (281). That the activity of ENaC was a critical contributor to the development of hypertension was graphically illustrated by the linkage of Liddle's syndrome, a rare inherited disease characterized by extreme hypertension (245), to mutations in ENaC subunits (350). Ultimately, it was found that deletion of a proline/tyrosine (PY) motif in the intracellular COOH-terminal tails of ENaC prevented binding of a ubiquitin ligase termed Nedd4-2 (1, 152, 165, 166, 262, 343, 361). As a consequence, the channel subunits were poorly ubiquitinated and the channel complex was not appropriately internalized, increasing channel residence time at the plasma membrane (115). This increase in N results in increased Na+ reabsorption and impressive hypertension (245). Thus the activity of Nedd4-2 is no longer able to bind, and channel is retained at the cell surface (301).

In addition to affecting channel number at the membrane, mutations can also have direct effects on channel activity causing increases in channel open probability (21, 129). Since this early work, multiple mutations have been identified in all three subunits of ENaC, some of which are associated with the development of hypertension. Whereas Liddle’s syndrome was initially determined as being due to two premature stop mutations [ENaC R564stop and ENaC W574stop (165, 166, 342, 343, 350)], it is now known that point mutations in the critical proline-rich regions of the carboxyl termini, e.g., in ENaC P615H, P616R/L/S, P617L, Y618H (138, 166, 198, 213, 329, 381, 390), are additionally associated with the disease. Mutations outside this region in ENaC have also been related to inherited hypertension, e.g., E607stop (199), as have polymorphisms, e.g., T594M (26, 305, 376) although the strength of this latter linkage has been questioned (284). A more recent study has gone one step further and identified multiple single nucleotide polymorphisms in all three ENaC subunits that are associated with variations in blood pressure due to changes in dietary salt intake (440). In contrast, some mutations in ENaC are associated with the complementary autosomal recessive condition known as pseudohypoaldosteronism Type I or PHA1. As the name implies, the hallmarks of this condition are salt wasting, hypotension, and hyperkalemia and can be due to loss of function mutations in any of the three ENaC subunits (49, 77, 323, 340, 366).

<table>
<thead>
<tr>
<th>Therapeutic Indication</th>
<th>ENaC Subunit</th>
<th>ASIC Subunit</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angina</td>
<td>x</td>
<td>x</td>
<td>(193, 422)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>x</td>
<td>x</td>
<td>(409, 411)</td>
</tr>
<tr>
<td>ARDS</td>
<td>x</td>
<td>x</td>
<td>(91, 189)</td>
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<tr>
<td>Autoimmune encephalitis</td>
<td>x</td>
<td>x</td>
<td>(132)</td>
</tr>
<tr>
<td>Bladder outlet obstruction</td>
<td>x</td>
<td>x</td>
<td>(13)</td>
</tr>
<tr>
<td>Central modulation of pain</td>
<td>x</td>
<td>x</td>
<td>(32, 108, 272, 347, 426)</td>
</tr>
<tr>
<td>Colonic carcinoma</td>
<td>x</td>
<td>x</td>
<td>(293)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>x</td>
<td>x</td>
<td>(12, 53, 61, 87, 178, 179, 228, 312)</td>
</tr>
<tr>
<td>Deafness</td>
<td>x</td>
<td>x</td>
<td>(159, 177)</td>
</tr>
<tr>
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<td>x</td>
<td>x</td>
<td>(172)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>x</td>
<td>x</td>
<td>(45, 259, 338, 443)</td>
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<tr>
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<td>x</td>
<td>x</td>
<td>(2, 424)</td>
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<tr>
<td>Glaucma</td>
<td>x</td>
<td>x</td>
<td>(113)</td>
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<tr>
<td>Glioma</td>
<td>x</td>
<td>x</td>
<td>(327, 396, 397)</td>
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<tr>
<td>Huntington’s disease</td>
<td>x</td>
<td>x</td>
<td>(415)</td>
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<tr>
<td>Hypertension</td>
<td>x</td>
<td>x</td>
<td>(360)</td>
</tr>
<tr>
<td>IBD</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Infertility</td>
<td>x</td>
<td>x</td>
<td>(74, 116, 229, 335)</td>
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<tr>
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<td>(298, 299, 374)</td>
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<td>x</td>
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<tr>
<td>Polycystic kidney disease</td>
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<td>(287)</td>
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<tr>
<td>Pulmonary infection</td>
<td>x</td>
<td>x</td>
<td>(91)</td>
</tr>
<tr>
<td>Retinitis pigmentosa</td>
<td>x</td>
<td>x</td>
<td>(122, 123)</td>
</tr>
<tr>
<td>Salivary gland carcinoma</td>
<td>x</td>
<td>x</td>
<td>(428)</td>
</tr>
<tr>
<td>Sjogren’s syndrome</td>
<td>x</td>
<td>x</td>
<td>(383)</td>
</tr>
<tr>
<td>Stroke</td>
<td>x</td>
<td>x</td>
<td>(418-420)</td>
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ENaC, epithelial sodium channel; ASIC, acid-sensitive ion channel; ARDS, acute respiratory distress syndrome; GERD, gastroesophageal reflux disease; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome.
However, the majority of hypertension cases have no such obvious cause as a gain of function mutation in ENaC. How then could hyperactivity of ENaC result? One way might be to increase trafficking of the channel to the membrane or to decrease its retrieval from the membrane as described above, more active surface channels resulting in increased Na\(^{+}\) absorption. An alternate possibility to increase the surface population of active channels could be to modify the channel on its way to the membrane or to activate silent channels already inserted into the membrane. Evidence has emerged over the past few years that ENaC undergoes a surprising degree of proteolytic processing (164, 222, 224). Initially, Vallet and colleagues showed that a serine protease called channel activating protease 1 (CAP1; prostasin), increased currents associated with ENaC in Xenopus oocyte expression systems (393, 400). Since then it has become clear that ENaC is cleaved and that two channel populations, one cleaved, one uncleaved, are trafficked to the cell membrane (224). Intracellular cleavage by furin residing in the trans-Golgi network cuts α- and γENaC, resulting in channels with substantially increased activity at the cell surface; how the uncleaved channel pool escapes this fate is currently unclear. Extracellular proteases, e.g., elastase and the channel activating proteases, also increase channel activity, and it has been proposed that this is due to the cleavage of the “silent” channel pool located at the plasma membrane (63, 187). In fact, in the absence of furin activity, channel open probability is fairly low (187, 224). Protease activity liberates endogenous inhibitory peptides from the extracellular loop of both α- and γENaC subunits (58, 67, 69, 70). Exogenous application of these inhibitory peptides to either natively expressed ENaC channels, or to channels expressed heterologously in Xenopus oocytes, was also capable of inhibiting the channel (67, 69, 297). These findings suggest novel antihypertensive therapies that include either protease inhibition or delivery of peptides to the luminal surface of the distal nephron to treat an overactive ENaC channel.

However, the number of channels can also be increased by affecting transcription or translation. ENaC expression is most closely associated with the mineralocorticoid, aldosterone. Serum levels of this hormone increase under conditions of sodium deprivation or potassium excess, and it is a key player in the regulation of whole body salt and water homeostasis. Aldosterone induces expression of αENaC in the collecting duct (255, 266), via a steroid response element in the 5'-flanking region of the αENaC gene (275). Both glucocorticoid and mineralocorticoid receptors can bind to this element, and both glucocorticoids and mineralocorticoids can bind to this site of the αENaC gene (275). Both glucocorticoid and mineralocorticoid receptors can bind to this element, and both glucocorticoids and mineralocorticoids can bind to this site of the αENaC gene (275). Both glucocorticoid and mineralocorticoid receptors can bind to this element, and both glucocorticoids and mineralocorticoids can bind to this site of the αENaC gene (275). Both glucocorticoid and mineralocorticoid receptors can bind to this element, and both glucocorticoids and mineralocorticoids can bind to this site of the αENaC gene (275).
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receptor (126, 134). Receptor overlap thus clearly allows for glucocorticoids to stimulate ENaC transcription. However, some degree of specificity is provided by 11-β-hydroxysteroid dehydrogenase type 2, which is highly expressed in aldosterone target tissues. This enzyme converts cortisol to cortisone (which is not a ligand for the mineralocorticoid receptor) and therefore limits the ability of glucocorticoids to activate transcription. In the kidney, the transcriptional effect of aldosterone is confined to the α-subunit; transcription of β- and γ-subunits is not affected (15, 120, 288, 365). In contrast, in the lung and colon, transcription of the β- and/or γ-subunits appears to be the rate limiting step (15, 291, 380). Additionally, ENaC transcription in the lung is under developmental control, reflecting the phenotypic switch from a secretory to an absorptive epithelium that occurs at birth (139, 170, 356).

ENaC transcription is also subject to repression, and an additional role of aldosterone is downregulation of a histone methyl-transferase termed Dot1a and its binding partner A9. Knockout of either protein significantly increased activity of the αENaC promoter (437, 438). Serum-glucocorticoid kinase 1 (SGK1), which regulates multiple effector proteins including ENaC (95, 124, 237, 302), regulates transcription of αENaC by phosphorylating A9, preventing the A9/Dot1a interaction and consequently increasing αENaC expression (439); Dot1a, in turn, downregulates expression of SGK1 (320, 435), and downregulates 11-β-hydroxysteroid dehydrogenase thereby increasing the responsiveness of tissues expressing the mineralocorticoid receptor to glucocorticoids (276). There may also be a role for αENaC splice variants in the expression of the full-length ENaC subunits, as increased transcription and translation of shortened splice variants may underlie the rescue of Dahl salt-resistant rats from the deleterious consequences of a high-salt diet by interfering with normal channel assembly (344, 345). Interestingly, expression of ENaC subunits is greater in Dahl salt-sensitive rats than in the Dahl salt-resistant rats, and is further increased when these animals are placed on a high-salt diet (9, 11). Epigenetic mechanisms such as these are increasingly being appreciated as having an important role in regulation of hypertension and ENaC expression, and may well underlie differences in tissue responsiveness to aldosterone stimulation (42, 52, 435).

Perhaps the singular most defining characteristic of ENaC is its sensitivity to the diuretic amiloride. This drug blocks the channel at submicromolar (IC50 ~0.1 μM) concentrations and is described as a K+-sparring diuretic as it inhibits ENaC-mediated Na+ absorption which reduces the driving force for K+ secretion, preventing the loss of K+ as seen with thiazide and loop diuretics. Although amiloride is highly effective, it is not widely used as a single therapy due to its potential to cause life-threatening hyperkalemia in patients with compromised ability to secrete K+; most current therapies for hypertension combine amiloride with a K+- wasting thiazide or loop diuretic or target the renin-angiotensin system and vascular smooth muscle. However, one situation in which amiloride has been proposed as an appropriate therapy is in patients with refractory hypertension. In one clinical trial, amiloride significantly reduced systolic and diastolic blood pressure (334) without causing hyperkalemia, and a case has been made that ENaC hyperactivity may underlie resistant hypertension (72, 313).

In marked contrast to hypertension, cystic fibrosis is a monogenic disease due to mutations in the cftr chloride channel (324). The classical manifestations of CF are the presence of thick sticky mucus in the airways due to poor surface hydration as a result of impaired apical Cl- secretion, a high sweat salt concentration, and poor pancreatic enzyme secretion. Experimental evidence suggests that hyperactivity of ENaC also contributes to reduced airway fluid clearance by increasing Na+ absorption and further reducing fluidity of airway mucus, a relationship first noted in CF epithelia prior to the identification of either the CFTR or ENaC genes (50, 225). An association between CFTR and ENaC was first reported by Stutts et al. (367), who showed that amiloride-sensitive Na+ transport was reduced in cells expressing both channels, whereas expression of ENaC alone was associated with large inward currents. These data suggested that wild-type CFTR exerted an inhibitory effect on ENaC to reduce ENaC P0, a finding that has been replicated by several groups (201, 241, 251, 368).

The mechanism underlying this functional interaction is, however, controversial, having been variously attributed to changes in intracellular chloride affecting ENaC activity (25, 54, 230), through changes in electrical driving force (73, 181, 202), to technical issues surrounding efficiency of voltage clamping (282). CFTR and ENaC may directly interact, based on evidence obtained from planar lipid bilayer and fluorescence resonance energy transfer (FRET) studies (37, 39), and from studies demonstrating that wild-type, but not ΔF508-CFTR, protects ENaC from proteolytic cleavage, thereby limiting the number of channels in the highly active cleaved ENaC pool (147). Wild-type CFTR may also restrict the activity of ENaC by limiting ENaC expression and trafficking to the surface, an effect that was not observed in cells overexpressing ΔF508-CFTR (331). However, it has been recently reported that αENaC is expressed on the surface of motile cilia in the airways, which would suggest limited opportunities for ENaC to interact with a CFTR channel located on the apical cell membrane (116). The presence of multiple potential mechanisms likely reflects the number of different experimental models that have been adopted to address the problem, species variation, and the tendency of cells to change their repertoire of expressed proteins as they adapt to culture conditions (432). It should also be noted that sequencing of ENaC in patients with CF or unexplained CF-like disease revealed multiple mutations in all three ENaC subunits, some of which were associated with hypo- or hyperchannel activity (22, 346).

However, uncertainty as to the exact mechanisms of CFTR/ENaC interaction has not limited the exploration of ENaC as a therapeutic target in CF. Amiloride used in an aerosolized formulation in short-term clinical trials for CF improved mucociliary clearance (12, 228). However, long-term trials were disappointing, with little improvement in several parameters of lung function (312), and it became evident in the trials (61) that the rapid clearance and relatively low potency of amiloride contributed to its poor performance. Two amiloride derivatives, benzamil and phenamil, were little better, despite having improved solubility and potency (178). A third generation amiloride analog, 552-02, exhibiting greater potency, longer channel interaction time, and slower lung clearance markedly improved mucociliary clearance, but more extensive trials will need to be conducted to determine effects of this compound on lung function (179). A second ENaC-related strategy has been to use protease inhibitors in an attempt to decrease ENaC

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cleavage and thus reduce the potential hyperactivity (53, 87). One of these compounds, camostat, was associated with increased mucociliary clearance in sheep airways, but more extensive lung function tests have not been reported; interestingly, the effectiveness of this agent in reducing ENaC activity has led to its consideration as a novel therapy for salt-sensitive hypertension (260).

**ENaC in non-CF-Related Lung Disease**

Apart from regulating the depth of the airway surface liquid layer that is reduced in cystic fibrosis, the main role of sodium channels in the lung is to maintain a relatively dry environment in the alveolus to facilitate gas exchange. All three main ENaC subunits are expressed in both type I alveolar cells [AT1, involved in defense against oxidative injury (43, 212)] and type II cells [AT2, responsible for surfactant secretion (211, 380)]. There is a significant upregulation in αENaC expression in the alveoli around birth, consistent with the switch from the fluid-filled uterine to an air-breathing environment (408). Knockout studies demonstrated that mice lacking αENaC expression failed to clear the liquid that filled the lungs during gestation, dying within 40 h of birth (188). Upregulation of ENaC also provides a rationale for the treatment with corticosteroids of neonatal respiratory insufficiency seen frequently in preterm infants (29, 171, 242). Interestingly however, patients with PHA-1, who have poorly functioning ENaC, do not present with neonatal respiratory distress syndrome (RDS), but develop lung disease a few months after birth (189). Outside of the neonatal period, poorly functioning ENaC is also associated with the development of pulmonary edema (269). This condition can be caused by multiple events but is most commonly associated with increased pressure in the pulmonary vessels and/or acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). Reduced ENaC activity predisposes the lung to flooding as a consequence of increased hydrostatic forces or hyper- or hypoxia (189). In contrast, mice that expressed the βENaC Liddle’s mutation, i.e., a hyperactive Na⁺ channel, were protected from the deleterious consequences of hydrostatic edema evoked by volume overload (318). One potential therapeutic strategy therefore could be to increase activity of ENaC in the alveolar epithelium. However, although there is much experimental evidence to support increasing ENaC NPs, in the alveolus using β-adrenergic receptor agonists (130, 244, 304), recent phase III trials with albuterol have been disappointing (268). One reason for this may be decreased responsiveness of alveolar β-adrenergic receptors as a consequence of cytokine release, hemorrhagic shock, and/or infection (93, 278, 386). The picture is further complicated by the presence of more than one type of amiloride-sensitive channel in alveolar epithelium. A recent study by Lazrak et al. (239) showed that two amiloride-sensitive channels, with single-channel conductances of 4.5 pS and 18 pS, could be recorded from AT1 and ATII cells in lung slices from air-breathing mice. The 4.5 pS channel was highly selective for sodium and most likely corresponds to αβγENaC, while the larger channel was less sodium selective and its molecular identity is currently unknown. Interestingly, however, activity of both channels was decreased by exposure to chlorine gas, which was matched by a decrease in αENaC expression.

ENaC is also thought to have a direct role in pulmonary infections. Several bacterial and viral pathogens have been found to inhibit sodium transport in vitro, including *Mycoplasma pulmonis* (176, 236), *Pseudomonas aeruginosa* (153, 369), *Mycobacterium tuberculosis* (436), SARS (208), respiratory syncytial virus (RSV) (82, 90), and influenza A (235, 240), although some of the findings reported in these studies have been harder to reproduce in vivo (143, 321). Several mechanisms have been proposed to account for the effects of the pathogens. Release of nucleotides such as UTP from the invading organisms, defending immune cells, or the epithelial cells themselves would inhibit ENaC (92, 196, 197, 263, 317). Binding of UTP to specific purinergic P2Y2 receptors in the lung has been proposed to couple to the PKC pathway, PKC being a known inhibitor of ENaC activity and expression (19, 91, 364). ENaC is also inhibited by reactive oxygen and nitrogen species (RONS), e.g., peroxynitrite, released during the process of lung inflammation (111, 160, 183, 283). In contrast, superoxide seems to enhance ENaC activity, and protects against the negative influence of RONS (114, 169, 431). The potential underlying mechanisms for the negative effects of peroxynitrite include posttranslational modification of vulnerable tyrosine and/or cysteine residues in the channel, although little direct evidence has been obtained to support this (81). The effects of superoxide are most likely due to modulation of NADPH oxidase, and scavengers/inhibitors of superoxide resulted in an acute decrease in channel Pₜ (379). This finding, which suggests that a tonic level of superoxide may be required for sustained channel activity, correlates with studies demonstrating that sustained hypoxia is associated with decreased expression of the three main ENaC subunits (384). Furthermore, pulmonary edema due to high altitude (i.e., low oxygen tension) was also associated with poor sodium transport (261). Conversely, sublethal (85%) hyperoxia increased activity of ENaC (433). Superoxide can also contribute to lung damage and the eventual failure of the respiratory epithelial barrier by reacting with NO to generate peroxynitrite (4) and nitrotyrosine, which are recovered from patients with ARDS (163), hantavirus infection, and SARS (91). Clearly, the lung must maintain a delicate balance between the positive and negative effects of free radicals to maintain an environment suitable for gas exchange—it is in cases of infection or injury that the balance tips in favor of RONS generation and decreased ENaC activity.

**Other Roles for ENaC in Vascular-Cardio-Renal Disease**

In addition to the contribution ENaC makes to hypertension, ENaC has also been implicated in other aspects of renal and cardiovascular disease. Several studies have suggested that ENaC subunits are expressed in vascular smooth muscle (205, 206), where they are thought to play a role in mediating myogenic constriction. While regulation is common to all vessels, it is of critical importance in the kidney, where vessel constriction in response to increases in pressure protects delicate nephrons and microvasculature from injury. Central to this response is the idea that ENaCs can act as stretch-sensitive channels, a concept originating with the finding that the ENaC family are homologs of a mechanosensitive family of proteins identified in *Caenorhabditis elegans* (64, 65, 264). It is tempting to speculate that the large extracellular loop characteristic

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of the Deg/ENaC family could be tethered to the extracellular matrix, while the cytoplasmic tails are linked to the cytoskeleton (Smith PR, unpublished observations); such an arrangement would provide a way to transduce force into channel opening (377). An alternate possibility is that direct stretch of the membrane evoked, for example, by cell swelling, could mechanically gate the channel. However, results from experiments designed to test this hypothesis have been contradictory (18, 20, 68, 207, 296). Another possibility is that flow-induced shear stress rather than membrane deformation activates the channel (5, 71), although in an oocyte-based assay it was found that shear stress did not play a part in channel activation (339), while in the intact nephron increasing flow decreased channel activity, most likely due to upstream release of inhibitory mediators (394).

The potential for a mechanosensitive role for ENaC has been most extensively investigated in the vasculature. Vascular smooth muscle cells express β- and γENaC subunits, but not αENaC (205, 206), and ENaC inhibitors, as well as knockdown of ENaC subunits, block myogenic constriction (106, 158, 205). The nature of the channel involved in this response is not entirely clear, but the lack of expression of the conductance ENaC subunit makes it unlikely that the channel involved is the “classical” αβγENaC channel of the renal collecting duct. It has been shown, that it is possible to form functional cross-clade hybrid channels composed of ENaC and ASIC subunits (273), and ASIC subunits have been identified in vascular smooth muscle cells where they play a role in cell migration (155, 156). Furthermore, myogenic constriction of the cerebellar artery was compromised in an ASIC2 knockout mouse model, suggesting that ASIC2 is intimately involved in this response (140). A more recent study has similarly proposed that an ENaC/ASIC2 hybrid may underlie mechanosensitivity in mammalian muscle spindles (352). Interestingly, a mechanosensitive role for ENaC has also been proposed in the urinary bladder, where the release of ATP from epithelial cells in response to increased hydrostatic pressure was prevented by amiloride (285). Furthermore, ENaC expression was found to be increased in the bladder mucosa patients with urinary outlet obstruction (13). Expression of βENaC also seems to be critical to the autoregulation of renal blood flow, as myogenic constriction was compromised in a murine model poorly expressing this subunit (105, 154). Thus, there is an emerging role for ENaC and the related ASIC subunits in vascular regulation.

ENaC subunits are also expressed in the nodose ganglia which receive inputs from arterial baroreceptors and it has thus been suggested that they are directly involved in sensing pressure changes transmitted from the aortic arch (107, 358), although amiloride did not block mechanosensitive currents evoked from nodose ganglia elicited by hypotonic solutions (102). ENaC may additionally play a role in the central control of blood pressure. The increase in blood pressure resulting from injections of hypertonic saline into the cerebral ventricle of rats was prevented by prior application of benzamil, suggesting that increases in sodium content of the cerebrospinal fluid may be sensed by ENaC, which is expressed in discrete locations throughout the cerebral cortex (10, 150, 378). However, here again, expression of γENaC was much more variable, suggesting that channels other than traditional αβγENaC may be involved in this response.

ENaC is also thought to be upregulated in polycystic kidney disease (PKD). The polycystic syndromes encompass a number of different genetic conditions in which the primary cilium is either shortened or absent. The most common manifestation is the development of fluid-filled cysts in the kidney and in some cases other tissues such as the liver, which is likely linked to a loss of epithelial polarity (413). PKD is associated with the development of severe hypertension; in the case of the recessive form of the disease, patients develop early and severe arterial hypertension and 20–45% will develop end-stage renal disease by their mid-teens (414). One cause of hypertension in these patients is likely due to unrestricted activity of ENaC. Studies in collecting duct cells derived from a murine genetic phenocopy model of PKD have suggested that sodium transport is upregulated, resulting in increased Na⁺ absorption that contributes to the development of hypertension (287). This finding would also be consistent with the low Na⁺ concentration of cyst fluid isolated from autosomal recessive polycystic kidney disease tissue (326).

Lastly, ENaC has been proposed to play a role in several nephropathies. Conditions such as diabetic nephropathy are associated with urinary protein loss and edema. They are also associated with increased sodium absorption in the absence of low blood volume, which would be predicted to increase sodium retention via the renin-angiotensin-aldosterone system (375). Increased proteinuria occurs due to glomerular damage, resulting in the escape of plasma protein into the tubular fluid; it can be both a consequence of, and a risk factor for, the development of hypertension. Owing to the enhancement of ENaC activity as a result of proteolytic cleavage, it has been proposed that the failure of the glomerulus to adequately remove plasma proteases from the glomerular filtrate results in increased ENaC activation with consequent increases in renal sodium absorption. One protease that has received interest in this context is plasmin, a protease cleaved from plasminogen by tissue plasminogen activator (TPA) and urokinase/α-PA. Plasmin is usually involved in fibrinolysis, providing a rationale for the use of TPA to reduce or prevent clot formation. However, levels of plasmin and plasminogen are elevated in the urine of nephrotic patients (395), while TPA and urokinase are both expressed in the kidney (233, 308). Recent evidence has shown that plasmin present in nephrotic urine can cleave γENaC, thereby activating the channel and increasing sodium absorption (298, 299, 374). Thus ENaC could be a reasonable target for therapy of nephrotic syndromes, as well as conditions such as preeclampsia (375).

**ENaC in the Peripheral and Central Nervous Systems and in Sensory Perception**

In addition to its role in Na⁺ transport in epithelia and in the vasculature, ENaC is also expressed in the nervous system and in sensory organs. Fungiform, vallate, and foliate taste buds all express ENaC subunits although expression of β and γENaC is much lower in the vallate and foliate buds (185, 231, 248, 250). This corroborated and expanded on earlier work which established that amiloride significantly attenuated the gustatory transduction of sodium (168). In mice, knocking out the conductive αENaC subunit from taste receptor cells abolished the sodium taste response (76). However, the effect of amiloride to block salt taste is not uniform, which may be explained by the.
presence of single nucleotide polymorphisms in the primary sequence of αENaC (127, 348). These studies suggest that a small-molecule potentiatior or activator of ENaC (256) could help with adherence to a low-salt diet, by helping recreate the sensation of higher sodium intake through manipulation of the channel.

ENaC is also involved in auditory sensation where both its role in fluid transport and potential mechanosensitivity are implicated. An antibody directed against the renal sodium channel complex decorated the tips of the stereocilia of the cochlear hair cells, which is the main location for the mechanoelectrical transduction or MET channel (137, 161, 162). Furthermore, amiloride blocked mecano-electrical transduction in cochlear cells in culture, and bound to tips of the cilia, suggesting that a Deg/ENaC subunit may be a component of the mechanotransducer (136, 333). However, mice in which αENaC has been knocked out exhibited no defect in hair cell mechanotransduction (332), and patients with PHA1 do not exhibit hearing loss (306), suggesting that the MET channel is not synonymous with αβγENaC.

ENaC is involved in fluid transport in the inner ear, being important for maintaining a much lower sodium concentration in the endolymph in the sacculate (220, 221), and the semicircular canals (310, 311), as compared with that in the perilymph. These findings are of relevance for conditions such as Meniere’s disease, where an increased volume of endolymph underlies the dysregulation of balance and symptoms of vertigo experienced by those afflicted. ENaC serves the same absorptive function in Reissner’s membrane, which separates the endolymph of the scala media from the perilymph of the scala vestibuli of the cochlea (243). Interestingly, a membrane-bound serine protease that is mutated in a form of inherited deafness also activates ENaC, consistent with the experimental studies indicating a role for this channel in auditory perception (159).

The role of ENaC in other senses (vision, touch, smell), is less well established. ENaC subunits have been detected in retina and in Muller glia (55, 56, 148, 149, 277), and amiloride affects multiple components of the electroretinogram (55). In a mouse model of glaucoma, neuronal retinal expression of αENaC increased, while that of β- and γENaC decreased (113). However, the exact role of the channel in the retina requires further investigation. Both β- and γENaC (but not αENaC) have been detected in dorsal root ganglia neurons and in the specialized organs that are responsible for the detection of touch in the skin, but the exact molecular nature of the channel involved is not known (104). All three ENaC subunits have also been detected in trigeminal sensory neurons, but their exact role similarly remains unclear (131). ENaCs may be involved in sensing temperature changes, as cold was found to increase ENaC currents, but had little effect on the currents associated with ASIC expression in the absence of low pH (16). ENaCs have additionally been identified in nerve endings in the larynx and in the nodose ganglia, where they may be involved in both mechanosensitivity and chemical sensitivity detected by laryngeal taste buds (423). ENaC is also a well-established member of the channel repertoire of the nasal epithelium, but this tissue is predominantly involved in fluid transport, and is widely used in CF-related studies as a model of the airway epithelium. To date, the only member of the Deg/ENaC family that has a direct role in olfaction is the Drosophila homolog pickpocket, which is thought to be involved in sensing of female pheromones by male flies (247).

ENaC in the Gut and Reproductive Systems

The only segment of the gut proper that exhibits appreciable expression of ENaC is the distal colon. Unlike other regions in the gut, the colonic lumen contains relatively little sodium, making a diffusional pathway an inappropriate mechanism for Na+ transport. As was ably demonstrated by Canessa et al. (64) in the original studies reporting the cloning of ENaC, channel expression is increased in response to low dietary sodium and is responsive to aldosterone which increases expression of the β- and γ-subunits (117, 133). Because of its location in the colon, ENaC has come under increased scrutiny as to its role in inflammatory diseases such as ulcerative colitis (125). Inflammatory mediators such as IL-1β, TNF-α, and IFN-γ downregulate expression of β- and γENaC (8, 265), reducing sodium and fluid absorption, leading to profound diarrhea. A murine model of ulcerative colitis, the IL-2 knockout mouse, also exhibited reduced expression of β- and γENaC (30). Inhibition of ENaC-mediated sodium absorption was also reported even for noninflamed colon obtained from biopsies of patients with Crohn’s disease, which was attributed to TNF-α activation of the ERK pathway (434). The rotavirus enterotoxin NSP4 similarly inhibited electrogenic Na+ absorption in the colon (292). The underlying mechanism in this instance is less clear, but may involve endoplasmic reticulum calcium release with subsequent channel inhibition, or disruption of lipid rafts incorporating ENaC.

Expression of α-, β-, γ-, and δENaC subunits has also been reported in the esophagus (17, 424). Given this location, it has been proposed that the acid-sensitive δENaC may be involved in sensing low pH as experienced during heartburn (424). However, ASIC channels are also expressed in the esophagus (2), and it is likely that these channels are activated either of, or in addition to, ENaC. The oral mucosa also expresses an amiloride-sensitive short-circuit current that is most likely due to ENaC activation (289). In this location, ENaC activity would be associated with decreased oral hydration. This is the rationale underlying the use of the amiloride analog 552-02 in the treatment of xerostomia due to Sjogren’s syndrome; the results of Phase I trials have been promising, with 552-02 improving oral hydration, speech, and the ability to eat, but the results of further trials are awaited (383).

The potential role of ENaC in the endocrine system has not been extensively studied, most work focusing on the effect of hormones on ENaC. As described above, ENaC expression and activity is tightly regulated by aldosterone, and vasopressin. However, insulin is also an important and acute regulator of ENaC activity, increasing membrane trafficking of the channel via a phosphatidylinositol 3-kinase-dependent mechanism (319, 385). It has also been suggested that growth hormone and IGF-I can increase activity of renal ENaC, which in turn contributes to the volume expansion observed in patients with acromegaly (217). Consistent with this hypothesis, it was found that ENaC activity was normalized following treatment of the underlying acromegaly (216).

Of the remaining major organ systems, the one that provides the best target for ENaC-mediated intervention is the reproductive system. Developmentally regulated amiloride-sensitive
sodium transport was identified in blastocysts by Benos and collaborators in the early 1980s (35, 325), where it plays a role in the expansion of the blastocoele. Expression of all three main ENaC subunits have since been identified in a trophoblast cell line (96). ENaC is also expressed in the female reproductive tract. The main task of the tubal epithelium is maintenance of an appropriate fluid environment for sperm motility, capacitation, and, ultimately, fertilization of the oocyte. The uterine lining is also responsible for secreting fluid that is favorable for early development and ultimate embedding of the fertilized oocyte into the uterine wall. In situ hybridization studies in mice revealed that all three ENaC subunits were expressed in the surface epithelium throughout the lower part of the female genital tract (cervix, vagina), and it has recently been shown that αENaC is also expressed on the cilia lining the oviduct (75, 116). In the uterus, only αENaC was detected, and expression varied with the estrus cycle and inversely to the expression of CFTR (74). Increased fluid absorption during periods when ENaC expression is increased may be required to facilitate blastocyst implantation (335, 427). Postimplantation, uterine expression of ENaC gradually declines (74). Sperm also seem to have a basal activation of an amiloride-sensitive Na⁺ channel. Block of this channel resulted in hyperpolarization of the cell membrane potential, which is required for capacitation (173). These authors found that sperm expressed αENaC in the midpiece of the flagellum and δENaC in the acrosome. This latter subunit has also been identified in the testis (406); testis also expresses a unique ASIC-related member of the Deg/ENaC family (200). Although the molecular composition of the channel expressed in the reproductive tract still needs to be determined, expression of ENaC in reproductive tissues does provide a potential target for manipulation of fertility and in vitro fertilization procedures. One recent study reported that, in semen samples obtained from patients with asthenospermia, an amiloride analog (ethyl-isopropyl amiloride) caused a twofold increase in sperm motility (229).

Other Tissues

Several other tissues and cell types have also been found to express ENaC. For example, ENaC has been reported to play a role in antibody secretion from lymphocytes (441), to act as a mechanotransducer in osteoblasts (223), and to be required for correct differentiation of keratinocytes and formation of the epidermal barrier (57, 78). Whether or not ENaC can be harnessed as a drug target in these situations remains to be determined.

ASICs as Therapeutic Targets

The acid-sensitive ion channels or ASICs form a distinct branch of the Deg/ENaC superfamily of sodium channels. Four ASIC genes have been identified in humans, numbered 1–4, with a number of splice variants encoding a total of nine unique proteins (3, 24, 79, 144, 157, 253, 316, 404, 405). The channels formed by the ASIC proteins are proton-gated cation channels, opening rapidly in the presence of extracellular acid. Their fundamental role appears to be acid transduction, converting an acidic extracellular environment into a cellular signaling event, by allowing for the conduction of cations into the cell (232). In vitro, these channels activate in the pH range of 3.0–7.0, with homomeric ASIC1 channels having an EC₅₀ at about pH 6.0 while homomeric ASIC2 channels show an EC₅₀ at about pH 4.0 (219). Although ASIC-containing channels are primarily sodium conductors, the permeability ratio PNa⁺/PCa²⁺ for homomeric ASIC1a has been calculated to be relatively low compared with ENaC, with estimates of 2.5–18.5 (33, 405). ASICs form homomorphic and heteromeric homoclade channels, all of which have distinct kinetic and activation profiles. In general, ASIC3 is the most sensitive to increases in proton concentration, with a half-maximal pH of activation of pH 6.4 (174). ASIC2 is the least sensitive, requiring exposure to pH 4.5 for half-maximal activation. ASICs have been detected throughout the nervous system, specifically in areas of high synaptic density, localizing primarily to the soma and processes of neurons (6, 219, 411). This expression pattern has suggested that these channels play a role in sensory perception and various neural processes (252, 421). However, ASICs are increasingly being detected in nonneuronal settings including cancer cells (40), bone (203), intestinal and bladder epithelium cells (103, 337), and smooth muscle (156, 226, 337).

Although the ENaC and ASIC proteins are part of the same family and have similar functions, there are considerable differences between the proteins and assembled channels. At the functional level, the primary difference is one of activation; ENaCs are constitutively active or modified by proteases to become active while ASICs are gated by a drop in extracellular pH (219). More precisely, ENaCs exhibit open and closed states, while the ASICs exhibit those states with an additional inactivated or desensitized state where the channel stops or attenuates conduction despite continued stimulation by low pH in the extracellular milieu (219, 273). This difference has consequences for channel regulation; whereas control of ENaC function is primarily determined by the number of channels at the surface, trafficking may be less critical for ASICs as the channels are to some extent ligand gated. Mutation of homoclade ASIC channels to become constitutively active leads to cell death (407). Another important difference is the presence of naturally occurring peptide toxin inhibitors of homoclade ASIC-containing channels, which are not found for homoclade ENaC-containing channels (100, 119), although there is the peptide self-inhibitory domain in some ENaC subunits that is released by proteolytic cleavage (69). ASIC1 is also subject to proteolytic cleavage, although in this case, protease activity decreases current and shifts the pH sensitivity, rather than activating the channel (85, 309, 370, 401). However, a serine protease inhibitor, nafamostat, does inhibit currents due to ASICs 1, 2, and 3 (391), although as ASICs 2 and 3 are not subject to proteolytic cleavage, the underlying mechanism is unclear. ASICs are also sensitive to amiloride and related compounds; however, the IC₅₀ values for these drugs are around two to three orders of magnitude greater for ASIC channels as compared with the prototypical renal ENaC channel. In general, much less is known about ASIC regulation than about that for ENaC. Despite this, there are a number of therapeutic opportunities afforded by manipulation of ASIC protein function or expression, including pain states, psychiatric disorders, neurodegenerative diseases, and cancer (218, 412, 421, 428). The studies detailing the involvement of ASICs in these states come primarily from genetic knockout and inhibitor studies in animal models, though some human data are available.
**ASICs and Pain**

One of the chief indications for a targeted ASIC-based therapy is in the treatment of pain. The ASIC proteins as well as their characteristic acid-induced currents have been found in many cells of the peripheral nervous system (219, 412). It is also known that acidosis accompanies inflammatory and pain states, and recently a peptide toxin isolated from the Texas coral snake, the venom of which causes intense pain, has been found to be an agonist at ASIC1 and ASIC2 channels (47). These findings suggest that ASICs are involved in nociception as transducers of painful stimuli. It had been well established, with initial work from the late 1920s, that protons and acidic solutions caused painful sensations when rapidly applied (146). Identification of the ASICs and their presence in nociceptive neurons and in dorsal root ganglia rapidly led to their being considered as potential pain transducers (7, 286, 387, 399, 403). However, the interpretation of numerous studies investigating a role for ASICs in the transmission of acute cutaneous pain is complex, as there are differences between rodent models. Furthermore, knockout studies in rodents did not reveal loss of sensitivity to pain (80, 294, 314, 315), while in some cases, ASIC knockout actually increased the sensitivity to painful stimuli (80, 279). In contrast, several studies with blockers such as amiloride, its analog benzamil, and a novel non-amiloride-related compound, A-317567, have shown effects consistent with a role for ASICs; for example, the pain due to acid application or to heat could be blocked by these compounds (109, 392). Interestingly, NSAIDs such as ibuprofen and diclofenac, which seem to directly block ASICs in addition to their well-known effect on cyclooxygenase activity, also block cutaneous pain evoked by acid stimuli (214, 398). In addition to the compounds identified above, two specific peptide toxins, psalmotoxin (PcTx1), derived from a West Indian tarantula, and APETx2, derived from a sea anemone (100, 101, 118, 119), are highly specific blockers of ASIC1 and ASIC3, respectively. APETx2 is an effective blocker of pain due to arachidonic acid in rats, suggesting a role for ASIC3 in mediating pain due to inflammation. Importantly, this compound increases the pH sensitivity of ASIC3, such that this channel activates at higher pH (lower [H+]i), in the presence of arachidonic acid (99). In contrast, PcTx1, which blocked pain and activated the enkephalin pathway when injected intrathecally (272), was much less effective at blocking acute pain (99); however, ASIC1 may have a role in the formation of heteromeric ASIC 1+3 channels that are not sensitive to this toxin (98).

ASICs have also been implicated in pain sensations arising from skeletal muscle and joints, heart, lung, and the gastrointestinal (GI) tract. Neurons from the dorsal root ganglia (DRG) have a central role to play in mediating pain from the joints and muscles, and the presence of ASIC expression in these tissues is highly suggestive. ASIC3 knockout mice (ASIC3−/−) did not develop hyperalgesia of the paw consequent on carrageenan-induced inflammation of the gastrocnemius muscle, although the response to heat was maintained (354, 355), most likely due to the role of TRPV channels in mediating this pain modality. Similar results have been obtained in a localized in vivo knockout mouse model (402). Furthermore, ASIC3−/− mice did not exhibit the hyperalgesia in surrounding tissues associated with joint inflammation (secondary hyperalgesia) (191). ASIC3 expression was also increased in wild-type mice following joint inflammation (191, 192). It has been suggested that the presence of channel on afferent nerves following inflammation would be relayed to the spinal cord, which would then integrate increased sensitization into the behavior characteristic of secondary hyperalgesia, and that these findings suggest a role for ASIC3 in the pain associated with arthritis (191, 192).

In the heart, ASIC3 has been associated with pain due to angina and ischemic damage following cardiac arrest. Early studies indicated that the cell bodies of neurons going to the heart via the nodose and dorsal root ganglion responded to low pH; subsequently, it was demonstrated that the kinetics of this response were similar to those seen for ASIC3 (36, 373), although more recently it has been reported that those in the DRG more closely resemble currents associated with ASIC 2a/3 heteromers (167). These responses were triggered at relatively high pH values, which can be reached relatively swiftly in the early stages of cardiac arrest (373, 422). Lactate, produced under conditions of anaerobic metabolism as found in ischemia (194), increases the sensitivity of the channel to protons, by displacing calcium bound to an external high-affinity site (195). Recently, it has been recognized that ATP, which is also released as a consequence of ischemic damage, causes a leftward shift in the response of ASIC3 to acid (46). It has been proposed that this requires protein:protein interactions between ASIC3 and a second ion channel, the ionotropic P2X5 receptor, although whether this occurs between the channels directly or through an intermediary protein remains to be determined. Since these early studies, it has been recognized that ASIC3 is also found on sensory nerve endings innervating the vasculature, where they may serve to sense muscle ischemia (280).

Unlike the relatively small changes in [H+]i found in the heart, the GI tract and particularly the stomach experiences large excursions in pH during the course of the day. Gastric acid has a pH < 1 when secreted in response to a meal, although this is quickly neutralized in the gastric lumen to ~ pH 3–4 by buffers contained in food. In pathological conditions such as duodenal or gastric ulcer, and gastrolesophageal reflux disease, acid secretion can be associated with severe pain. In one study where gastric ulcers were induced in mice by short exposure of the gastric mucosa to acetic acid, gastric afferents in the DRG and nodose ganglia exhibited ASIC-like currents, which were attributed to expression of ASIC1 and ASIC2 subunits, based on the response to inhibitors (372). Signaling along the brain-gut axis was also disrupted in ASIC3 knockout mice which had been exposed to iodoacetamide to induce a mild gastritis (416). ASICs also likely contribute to the sensation of heartburn experienced by many individuals as a consequence of acid washed through the lower esophageal sphincter into the esophagus (2). ASICs 1, 2, and 3 have been detected in colonic neurons (186) and have been implicated in pain associated with inflammatory and irritable bowel syndromes. ASIC3 is upregulated in enteric neurons in the inflamed gut of patients with Crohn’s disease (430), while mRNAs for ASICs 1 and 2 were upregulated in the spinal cord of a rat model of irritable bowel syndrome; furthermore, intrathecal PcTx1 prevented the development of colonic hypersensitivity in this model (267). The role of ASICs in irritable bowel syndrome may be related to a role in sensing...
mechanical distension throughout the gut (295), although as with studies of cutaneous pain sensation, knockout studies are somewhat equivocal (330). In addition, gut ASICs have been postulated to regulate bicarbonate secretion in the duodenum (103). ASICs 2 and 3 have also been identified in pulpal afferents and have been proposed as therapeutic targets in dentin sensitivity (172).

Although ASIC3 seems to predominate in the mediation of pain signals from the periphery, ASIC1 or composite ASIC1/2 channels may have a more important role in pain sensation in the central nervous system (CNS). ASICs are expressed in the CNS in multiple locations including hippocampus, hypothalamus, midbrain, and cerebellum (6, 215, 274, 307). While not all of these locations are involved in pain sensing, it is likely that ASICs identified in neurons of the dorsal horn are responsible for integrating peripheral pain signals before transmission to the cortex (32). The finding that PcTx1 and ASIC1 antisense oligo-deoxynucleotides infused into the intrathecal space both had analgesic effects further suggests a role for ASIC1 in the central mediation of pain (108, 272). Moreover, recently defined interactions between ASICs and peptides found within the CNS, such as the dynorphins, reinforce the possibility that CNS ASICs may be manipulated to manage pain (347, 426).

In general, and despite data from knockout animals, a reasonable case can be made for ASICs, particularly ASIC1, being attractive targets for novel analgesic strategies. Acid-induced pain in humans was attenuated by treatment with amiloride (392), suggesting that inhibitors of ASICs, potentially including peptide toxins and aminoglycoside antibiotics (145), in addition to the more conventional amiloride and NSAID analogs (128), could play a role in the treatment of pain, while avoiding the behavioral issues associated with opiates (272). A more detailed account of the literature surrounding the role of ASICs in pain is provided in an excellent recent review (98).

ASICs and Psychiatric Disorders

Studies of ASIC1 in mice have implicated it in synaptic plasticity, learning, and memory formation (409–411). In the CNS, the protein appears to localize with the postsynaptic density-95 protein (PSD-95), which likely targets ASIC1 to areas of high synaptic density (409, 410). Behavioral tests of mice overexpressing ASIC1 found increased acquired fear related behavior (411), while ASIC1 knockout mice showed a deficit in cued and contextual fear conditioning (409), suggesting that perhaps ASIC1 could play a role in anxiety or fear learning. Interestingly, it has recently been reported that decreased brain pH resulting from hypercarbia also elicits fear in mice, a behavioral response that is attenuated by ASIC1 knockout (442). However, a case-control twin study showed no association between polymorphisms of ASIC1 and anxiety spectrum disorders (175). Still, functional tests with ASIC inhibitors in animal models found that ASIC inhibition was able to produce antidepressive, sedative, and anxiolytic effects (88, 112, 234). For example, amiloride, PcTx1, and A317567 all exhibited anxiolytic activity, with PcTx1 and A317567 being most effective, most likely reflecting the lower potency of amiloride in ASIC inhibition (112). The exact role and mechanism of ASIC in anxiety and fear learning remains to be elucidated, but the data suggest that inhibition of ASICs can play a role in the treatment of anxiety or depression states.

ASICs and Neurodegenerative Disorders

ASIC proteins in the CNS have been shown to play a role in a handful of neurodegenerative disorders (421), including ischemic stroke (419), Parkinson’s disease (14, 209), epilepsy (45, 259, 443), Huntington’s disease (415), and autoimmuneencephalitis (132). The mechanism by which ASIC proteins play a role in these pathologies varies and is still under study.

For example, during ischemic stroke there is a localized reduction in the extracellular pH. This acidosis is hypothesized to activate homomeric ASIC1 channels, leading to elevations of intracellular calcium and cell death. As noted earlier, the $P_{\text{Na+}}/P_{\text{Ca2+}}$ for homotrimeric ASIC1a has been calculated as 2.5 by one group and as 18.5 by another group (33, 405). The divergence between these two values is likely due to the difficulties with calculating the permeability for a channel that is allosterically modulated by the divalent cation in question as well as the innate kinetics of the channel, making it difficult to record accurate measurements (23, 38, 94, 142, 195, 300). While there is debate over the $P_{\text{Ca2+}}$ of homotrimeric ASIC1 channels, it has been shown that cells expressing homotrimeric rat ASIC1a can respond to an acidic pH pulse with an increase in cytosolic calcium (429). This increase is due to extracellular calcium influx, as it still occurs after the endoplasmic reticulum is emptied by thapsigargin, and is sensitive to amiloride (429). This increase in calcium is postulated to lead to increased cell death secondary to acidosis; cells expressing rat ASIC1a are more sensitive to cell damage by incubation in pH 5.0 medium than cells without the ion channel as measured by a lactate dehydrogenase release assay (429). This held true for COS-7 cells exogenously expressing rat ASIC1a as well as for hippocampal neurons natively expressing rat ASICs (429). However, heteromeric channels formed of ASIC1 and ASIC2 are calcium impermeable. In rat models, global ischemia upregulated ASIC2 expression which would theoretically increase the population of heteromeric ASIC1/ASIC2 channels, acting to protect neurons from further ischemic attacks (210).

Further in vitro models, as well as in vivo mouse and rat models of stroke have shown that inhibition of ASIC1 effectively protects neurons from acidosis or ischemia (141, 353, 418–420). In vivo animal data regarding ASIC1 suggest that inhibition of this channel will be effective up to 5 h after the stroke rather than the narrow one hour window of N-methyl-D-aspartate antagonists (353). However, there is some slight controversy to this calcium-based hypothesis, as Samways et al. (336) were unable to see a significant increase in intracellular calcium in physiological conditions in a large number of cells natively or exogenously expressing ASIC1. The authors observed increases in intracellular calcium in a small population of chicken dorsal root ganglia neurons, but the majority of the chicken DRG cells and the entirety of the HEK293 and COS7 cells did not show increases in intracellular $[\text{Ca}^{2+}]$ when expressing chicken ASIC1 or human ASIC1b (336). The difficulty in showing significant calcium permeability of ASIC1 channels, combined with the transient and desensitizing nature of ASIC1 currents, suggests that there may be more than a calcium conductance playing a role in the neuronal death during stroke.
Another mechanism of action was postulated for the role of ASICs in Huntington’s disease, where mutations in the huntingtin gene lead to the accumulation of a mutant protein with an expanded polyglutamine tract and neuronal damage (34). Studies using in vitro and in vivo models of Huntington’s disease showed that the use of amiloride or short hairpin RNA directed against ASIC genes was able to reduce the impact of the disease (415). The authors showed that reducing ASIC1 or ASIC2 function or expression led to an increase in the activity of the ubiquitin-proteasome system and showed a decrease in the toxic aggregation of huntingtin-polyglutamine (415). The chemical inhibitor and ASIC1 knockdown might suggest this could be due solely to calcium conductance capabilities of ASIC1, but similar results were found with knockdown of ASIC2, which is known to form calcium-impermeable channels (219, 415). Still, this work is promising and suggests a role for ASICs in this disease and possibly other CNS diseases where protein aggregation leads to neuronal death.

Epilepsy and Parkinson’s Disease

Increased activity of ASICs has been implicated in both epilepsy and Parkinson’s disease. Chemically induced seizures in rats are associated with a decrease in extracellular pH (351), likely due to efflux of lactic acid into the extracellular space. This raised the possibility that ASICs could be involved during seizure activity. Using pilocarpine to induce status epilepticus, Biagini et al. (45) found that the message level for ASIC2b was quickly (within 15 min), significantly, and persistently (sustained for at least 24 h) decreased in the hippocampus. This could not be accounted for by simple neuronal death as treatment with diazepam and pentobarbital, which protect neurons, did not prevent the loss of ASIC message. ASIC1a expression was also reduced, but in a more restricted area. A more recent functional study has demonstrated that ASIC1 activity is, however, important in seizure termination, as seizures in ASIC1 knockout mice were much more severe than in their wild-type counterparts; similar results were obtained when ASIC1 was acutely inhibited in wild-type mice by intracranial injection of PcTx1 (443). Conversely, overexpression of ASIC1 shortened seizure duration. Heteromorphic ASIC1a/2b channels are more sensitive to reductions in pH than either ASIC1a or ASIC2b alone (174) and reducing extracellular pH (pH₆) to 6.8 was sufficient to decrease seizure activity in hippocampal slices (443). Loss of ASIC2b would therefore be predicted to decrease sensitivity of a heteromeric channel to changes in pH₆. However, it is unknown whether heteromeric or homomeric channels are involved in seizure termination. Furthermore, seizure initiation and termination are highly complex processes, and the involvement of any channel type may differ based on the location of the seizure focus or even between species. Interestingly, expression of ASIC4 has been reported to be induced in pediatric patients with febrile seizures (338).

The involvement of ASICs in Parkinson’s disease is a relatively recent development. Parkinson’s disease is associated with neuronal degeneration, loss of dopaminergic neurons in the substantia nigra, and acidosis (51). In a mouse model of Parkinson’s disease, pretreatment of animals with amiloride or crude psalmotoxin venom prevented loss of dopaminergic neurons due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration (14). The effects of amiloride were comparable to those seen for other compounds capable of preserving dopaminergic neurons in the MPTP model. These results suggest the involvement of ASIC1 in neuronal loss, but the relative nonspecificity of the agents used suggests that these data should be interpreted with caution. However, a role for ASIC2a in Parkinson’s disease has emerged from studies of parkin, an E3 ubiquitin ligase that is mutated in genetically related autosomal recessive Parkinson’s disease. One target of parkin appears to be PICK1 (protein interacting with C kinase 1), a protein that binds to the COOH-terminal PDZ motif of ASIC1 and ASIC2 (110, 182), and which potentiates ASIC-related currents (31, 184). Overexpression of parkin suppressed ASIC2a currents in a heterologous COS-7 cell system, while OAG (1-oleoyl-2-acetyl-sn-glycerol, a PKC activator)-stimulated currents in hippocampal neurons from a parkin knockout mouse were greatly potentiated (209). In contrast, OAG had no effect on ASIC currents recorded from wild-type mice. These data suggest that hyperactivity of ASIC contributes to neurodegeneration in Parkinson’s disease.

ASICs and Sensory Perception

As with ENaCs, ASICs also have a role in sensory perception. ASICs are expressed in the retina, along with ENaCs (56, 271). Retinal ganglion cells, which integrate signals from the retina also express amiloride-sensitive proton activated currents, consistent with ASIC expression (246). Retinal neurons do experience pH fluctuations, and ASICs in this location may serve to modulate excitability. ASIC1a is found throughout the retina, including cones; inhibition with PcTx1 or knockout of ASIC1 using antisense oligonucleotides resulted in attenuation of the normal response to light as evidenced in the electroretinogram (121). In contrast, studies in ASIC2 knockout mice showed that this ASIC subunit sensitized the retina to light, and suggested that it had a negative effect on phototransduction (123). Consistent with this, the finding that ASIC2 knockout mice were more susceptible to light-induced retinal degeneration than their wild-type counterparts. ASIC3 is also important for maintaining retinal function, as knockout of this subunit similarly caused rods to die (122). Several features of the retinal damage found in these animals are similar to what is observed in human conditions such as Usher syndrome, retinitis pigmentosa, and glaucoma; whether or not there is a direct link to human disease remains to be determined. ASIC2 and ASIC3 are found in the inner ear, and although knockout animals do not exhibit obvious hearing deficits, knockout of these genes may increase susceptibility to noise (303, 330); for example, in the case of ASIC3 knockouts, hearing loss develop after the first four months of life (177). As might be predicted, ASICs are expressed in taste buds where they are, naturally enough, thought to modulate the modality of sourness (249, 254). However, there are some species differences, with mouse express ASICs 1 and 3, while in rat ASIC2 was also identified (254, 322, 349). However, mice are able to perceive sourness (322). Patients who could not detect sourness were also found not to express ASICs 1–3 in fungiform papilla, although, interestingly, expression of δENaC, the only acid-sensitive member of the ENaC family, was retained (190). Whether the presence of these ion channels can be exploited for the treatment of taste deficiencies remains to be seen.
Touch sensation also involves ASICs, although the exact role of the different channels is still somewhat unclear. Pacinian corpuscles express ASIC1 and 2 in humans (62), although loss of ASIC1 expression did not appear to affect touch sensation in mice (294). In contrast, ASIC3 has been suggested to be the important mediator of touch sensation in mice (315).

**ASICs, ENaCs, and Cancer**

One of the major pathologies for which a potential role of ASICs and ENaCs is currently emerging is cancer. Multiple studies have shown that ASICs and ENaCs are associated with cell migration and proliferation, even in nontumor cells (48, 83, 97, 155, 156). ENaC expression is also upregulated in melanoma cells (425), while in salivary gland carcinoma cells, an ASIC2/3 heteromeric conductance has been proposed to underlie a proton-gated current that was not present in normal salivary gland epithelia (428). To date, the best case for involvement of ASICs and ENaCs in tumors has come from studies of colon and brain malignancies. Using a genetic murine model of colon cancer, the heterozygous APCMin/+ mouse, Ousingsawat et al. (293) found that expression of αβγENaC was increased in the distal colon, which corresponded to an increase in sodium absorption. It was suggested that increased ENaC-mediated salt absorption would lead to decreased hydration of the colonic contents, thus leading to constipation, which has been proposed as a precipitating factor in the development of colonic carcinoma (89, 359). The increased ENaC expression and sodium absorption in this mouse were rescued by treatment with rapamycin, consistent with previous reports in lung epithelia and with the recently described role of mammalian target of rapamycin to increase ENaC currents via phosphorylation of SGK1 (227, 257, 290).

The involvement of ENaCs and ASICs in glioblastoma is somewhat more complex than their role in the previously described pathologies. Glioblastoma multiforme (GBM) are primary brain tumors of astrocytes or their progenitor cells. These tumors are characterized by high rates of invasion and dispersal throughout the brain. Tumor cells that migrate into the surrounding brain parenchyma escape surgical resection and are the source of recurrent tumors, accounting in part for the poor prognosis associated with this disease. Several members of the ASIC/ENaC family are expressed in astrocytes, cultured glioma cell lines and freshly resected tumor tissues. Messenger RNAs for ASIC1, ASIC3, γENaC, and δENaC are widely expressed in these cell types, while the expression of ASIC2, ASIC4, αENaC, and βENaC are more variable (40). More importantly, constitutively active, amiloride-sensitive sodium currents were also expressed in high-grade [World Health Organization (WHO) Grades III and IV] human glioblastoma primary cultures as well as in human glioma cell lines. This conductance was not present in lower-grade gliomas or in noncancerous astrocytes (40), and exhibited selectivity for cations (but was poorly selective between cations), was inhibited by PcTx1, but was not acid gated like a homo- or heteromeric ASIC-containing channel (60). Using a strategy adopted from studies in *C. elegans* (180, 205), it was observed that transfection of dominant negative constructs, which decreased expression of ENaC and ASIC subunits, was able to disrupt the glioma conductance, suggesting that this was a heteroclade channel formed through interactions between αγENaC and ASIC1 proteins (218). Further studies showed that a key difference between human astrocytes and glioma cells lay in the pattern of expression of ASIC2. Although ASIC2 was expressed in normal astrocytes and low-grade gliomas, ASIC2 mRNA (and protein) was absent in ~60% of high-grade glioma cells (40). Regardless of the presence or absence of ASIC2 mRNA, no ASIC2 protein was expressed in the plasma membrane of any glioma cell line tested (396). Electrophysiological studies of glioma cells showed that transfection of ASIC2, or increasing surface expression of ASIC2 by using glycerol and sodium phenyl-butyrate, inhibited the constitutively active, amiloride-sensitive, inward cation conductance (396). Later work showed that ASIC2 was specifically bound by Hsc70 and that knockdown of this chaperone was associated with increased surface expression of ASIC2 and loss of the constitutively activated current (397). Using an inhibitor of DNA methylation, 5-Aza-C, Xia et al. (417) were also able to increase expression of ASIC2 and rescue (i.e., inhibit) the current phenotype in those cell lines that did not express ASIC2 mRNA. Cell proliferation and migration assays carried out in these studies demonstrated that the translocation of ASIC2 to the plasma membrane inhibited the proliferation and mobility of GBM cells (396, 397). The observation that PcTx1 was a highly potent inhibitor of this conductance, and inhibited cell migration, proliferation, and volume regulation (327, 328), combined with the presence of these amiloride-sensitive currents in malignant cells and their absence in benign cells, suggests the possibility that these conductances may be useful therapeutic targets. Interestingly, several studies have shown that amiloride and its analogs reduce tumor growth and metastasis in rodent models of cancer, although the relative nonspecificity of this compound, especially at high doses where it inhibits Na+/H+ and Na+/Ca2+ exchangers and even a serine protease, makes it difficult to ascribe drug effects directly to ASIC/ENaC inhibition (see Ref. 270 for review).

**Summary**

As can be seen from the above discussion, ASIC and ENaC subunits are widely expressed. In the vast majority of tissues where expression has been found, it has been accompanied by the presence of amiloride-sensitive cation currents. However, not all of these currents conform to the small-conductance, high Na+ selectivity, high amiloride-affinity fingerprint associated with expression of αβγENaC in the kidney. Similarly, not in all instances where ENaC has been identified are all three of the main subunits expressed, and in several cases, ASICs are coexpressed with ENaC subunits. While environmentally mediated increases or decreases in expression of certain subunits, e.g., the response to circulating aldosterone levels and/or posttranslational modifications such as phosphorylation or carboxymethylation, account for some of the observed differences in amiloride-sensitive sodium channel characteristics, in most cases it is unknown whether expression of another subunit or other regulatory protein would alter the current profile. In addition, some ENaC and ASIC subunits, e.g., δENaC, ASIC4, have not been well studied and the roles of these subunits are poorly defined. Expression studies, especially those based solely on RT-PCR-based amplification of mRNA, should be interpreted with caution, given the ability of the technique to identify even vanishingly small amounts of
Given the wide expression of these proteins and their association with multiple diseases and conditions, it is hardly surprising that they form highly attractive targets for novel therapies. It has been proposed that a constitutively active degenerin mutant of ASIC2 could be delivered by virus for expression in tumor cells, provoking unregulated influx of sodium leading to rapid cell death (382). However, small molecules offer the most attractive therapeutic options, although currently available antagonists such as amiloride, benzamil, and NSAIDs suffer from lack of specificity or efficacy or have problems associated with delivery and availability such as the peptide toxins. For example, while amiloride is highly specific for αβγENaC, it has undesirable side effects, e.g., potassium retention, which can lead to the development of severe hyperkalemia in patients with compromised renal function. In doses sufficiently high to inhibit ASIC channels, amiloride is also a reasonable blocker of plasma membrane Na+/H+ and Na+/Ca2+ exchangers. More potent amiloride analogs with reasonable binding characteristics have been developed, but the results of clinical trials are equivocal. The overarching problem, therefore, is the identification (most likely by high-throughput screening) of appropriate small molecules that combine the desirable qualities of high specificity and efficacy with limited side effects in antagonizing these channels. However, further studies to assign molecular identities to recorded current and channel characteristics of this large and diverse family will also be required.

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AUTHOR CONTRIBUTIONS

Y.J.Q. and C.M.F. prepared the figure; Y.J.Q., A.K.R., and C.M.F. drafted the manuscript; Y.J.Q., A.K.R., and C.M.F. edited and revised the manuscript; C.M.F. approved the final version of the manuscript.

REFERENCES


73. Chabot H, Vives MF, Dagenais A, Grygorczyk C, Berthiaume Y, Grygorczyk R. Downregulation of epithelial sodium channel (ENaC) by CFTF co-expressed in Xenopus oocytes is independent of Gt" conduc-
102. Duggan A, Garcia-Anoveros J, Corey DP. The PDZ domain protein PICK1 and the sodium channel BNaCi interact and localize to mecha
103. DuVall MD, Zhu S, Fuller CM, Matalon S. Peroxynitrite inhibits amiloride-sensitive Na+ currents in Xenopus oocytes expressing aByr


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sodium channel is a key mediator of growth hormone-induced sodium retention in acromegaly. Endocrinology 149: 3294–3305, 2008.


Acid-sensing ion channels (ASICs) are a family of transmembrane proteins that are activated by extracellular acidification. They play diverse roles in various physiological and pathological states. One of the best-studied functions of ASICs is their involvement in ischemic brain injury. Blocking calcium-permeable acid-sensing ion channels (ASIC1a) in transgenic mice increases acoustic startle response, indicating an endogenous role for ASIC1a in fear behavior.

Acid-sensing ion channel 1 (ASIC1) is localized in brain regions with high synaptic density and contributes to fear conditioning. *J Neurosci* 23: 5496–5502, 2003.


