CMLS, Cell. Mol. Life Sci. 55 (1999) 1216–1229
1420-682X/99/101216-14 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

pH_i regulatory ion transporters: an update on structure, regulation and cell function

M. Pucéat[1,2]

[1]INSERM U390, Laboratoire de Physiopathologie Cardiovasculaire, C.H.U. Arnaud de Villeneuve, Montpellier (France), e-mail: puceat@crbm.cnrs-mop.fr
[2]CRBM, CNRS UPR 1086, Montpellier (France)

Received 30 December 1998; received after revision 4 February 1999; accepted 9 February 1999

Abstract. Intracellular pH (pH_i) is a major regulator of various and critical cellular functions. A close regulation of pH_i is thus mandatory to maintain normal cellular activity. To this end, all cells express ion transporters that carry across their plasma membrane H^+ or equivalent H^+ into and out of the cell. Besides pH_i, these ion transporters are under the regulation of neurohormonal stimuli. This review summarises the molecular identity, regulation and function of the main membrane pH-regulatory ion transporters.

Key words. pH_i; ion transporter; Na-H antiport; anion exchanger; NBC.

Introduction

Intracellular pH (pH_i) regulates many cellular processes, including cell metabolism, Ca^{2+} homeostasis [1], gene expression [2], cell motility and contractility, cell-cell coupling [3], cell adhesion [4] and cell death [5, 6].
Most mammalian cells feature a steady-state cytosolic pH (pH_i) of 7.1–7.2. Taking into account the metabolic activity, and the thermodynamic and resting membrane potential of a cell, and protons passively distributed across the plasma membrane according to the electrochemical gradient, pH_i should be much more acidic (around 1 unit pH lower than extracellular pH, which is roughly 7.3). For example, assuming a resting membrane potential of -80 mV for a cardiac cell, calculation using the Nernst equation gives an intracellular pH of 6.1 for a cell that would be bathed in an extracellular medium of pH 7.4. A pH_i of 6.1 would be incompatible with cellular processes such as metabolic pathways catalysed by pH-sensitive enzymes. Furthermore, the constancy of pH_i may at first glance be striking, as pointed out by Needham as early as 1926 [7]. To regulate and to maintain the constant value of cytosolic pH, the eukaryotic cells express at their plasma membrane protons pumps (H^+ ATPases) [8], proton channels [9] and ion transporters that drive H^+ or equivalent H^+ and HCO_3^- ions into and out of the cell. The latter include an Na^+/H^+ antiport [10], Na^+-dependent HCO_3^- transporters [11], an Na^+-independent Cl^-/HCO_3^- exchanger [12, 13] and the recently described Cl^-/OH^- exchanger (CHE) [14] (fig. 1). Other membrane transporters such as the lactate-proton cotransporter may also participate in cell pH regulation [15, 16].
The following review summarises the molecular identity, multiple regulation and functions of these pH_i-regulatory ion carriers and the forthcoming challenging issues of cell pH regulation.

Cell measurement of intracellular pH

Years ago, pH-sensitive electrodes built with H^+ exchange resins and impaled into tissues or cells were first

used to measure pH_i. Then, around 20 years ago, investigators used [^{14}C] 5,5-dimethyl-2,4-oxazolidione (DMO) that partitions across the membrane according to the pH gradient to monitor pH_i in suspensions of cells [17]. More recently, pH-sensitive fluorochromes, 4-methylumbelliferone (4MU), 2,3-dicyanohydroquinone (DCH), and 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF) and the most recent indicators carboxyseminaphthorhodafluor-1 (SNARF1) [18] and 5′(and 6′)-carboxyseminaphthofluorescein (SNAFL) [19] have been widely employed to monitor pH_i in single cells. These probes, synthesized as acetoxymethylester derivatives, are cell-permeant and have the property that they shift their light emission or excitation spectrum as a function of pH. Moreover, the excitation wavelengths of SNARF and SNAFL (around 500 nm) enable use of an argon/krypton laser to record pH using confocal microscopy [20]. Very recently, the pH sensitivity of green fluorescent protein (GFP), further enhanced by mutagenesis, was used advantageously to measure cytosolic pH. It further allows monitoring of pH of intracellular compartments, including the mitochondria and the Golgi apparatus, when GFP is targeted to the intracellular compartment of choice with a specific protein of the organite [21]. Combined with confocal microscopy, this technical approach is one of the most promising to understand the regulation of intracellular pH, taking into account not only plasma membrane ion transporters but also ion transporter of organites.

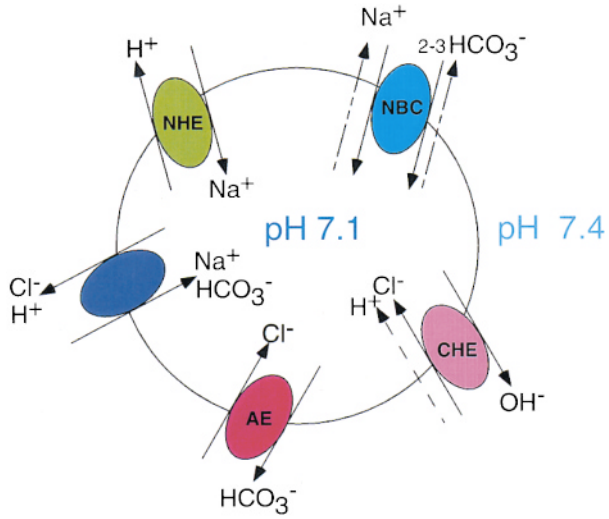

Figure 1. The pH regulatory ionic transporters. NBC, Na^+-dependent HCO_3^- cotransport; NHE, Na^+-H^+ antiport; AE, anion exchanger; CHE, Cl^-/H^+ exchanger.

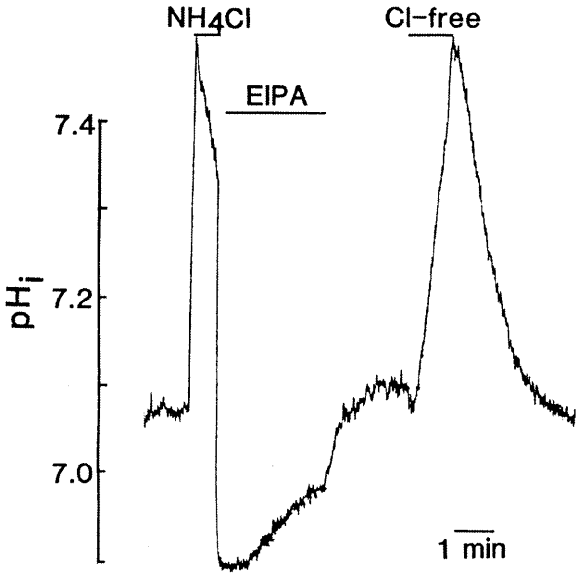

Figure 2. Functional pH regulatory ion transporters in cardiomyocytes. Cardiac cells loaded with Snarf-1 are bathed in an extracellular HCO_3^--containing medium. pH_i is monitored on a single cell [111]. After an acidic challenge triggered by removal of NH_4Cl, the pH recovery is mediated by a HCO_3^--dependent transporter in the presence of EIPA, an inhibitor of the Na-H antiport. The pH recovery is greatly accelerated when the Na-H antiport is functional after washout of EIPA. The Na^+-independent Cl^-/HCO_3^- (AE) is reversed in the absence of extracellular Cl^-, leading to an alkalinization, and works in its physiological acidifying direction when Cl^- ions are added back to the extracellular medium.

The proteins of the NHE family mediate Na^+/H^+ exchange

The Na-H antiport is an electroneutral ion exchanger that pumps H^+ ions out of the cell when it is turned on by acidic challenge imposed on a cell (fig. 2). The H^+ efflux is driven by the inwardly directed Na^+ electrochemical gradient. First discovered in kidney and intestine [10], the antiport has now been found in most tissues of eukaryotic cells. The diuretic amiloride and its chemical derivatives (dimethyl- or ethylsisopropylamiloride) are some of the most potent inhibitors of Na^+/H^+ exchange. The differential amiloride sensitivity of the Na^+/H^+ exchange [22] in various tissues predicted the existence of several isoforms of the protein that mediates this ion exchange. Indeed, Na^+/H^+ exchange is performed by different proteins that belong to the multigenic NHE (Na-H exchanger) family. So far, six members of the NHE family sharing 30% to 60% homology have been cloned. The first NHE gene (NHE1) was cloned by Sardet et al. [23] by gene complementation of an antiporter-devoid fibroblast cell line. NHE1, which displays the greatest sensitivity for

amiloride (EC_{50}: 3 μM for amiloride, 80 nM for methylpropylamiloride) or for the new Na-H inhibitor HOE694 (3-methylsulphonyl-4-piperidinobenzoyl) guanidine methanesulphonate; EC_{50}: 0.16 μM) [22] is a housekeeping gene. It is expressed in virtually all tissues. This is an N- and O-glycosylated protein [24] of 815 amino acids that migrate in SDS-polyacrylamide gel electrophoresis (PAGE) with an apparent molecular mass of 110 kDa. The NHE1 and NHE3 proteins expressed in the antiport-deficient cell line form stable dimers. However, individual subunits of NHE1 function independently within the oligomeric state [25]. Glycosylation of the protein does not affect the rate of ion transport [24, 26] but rather may play a role in antiport sorting. The antiport contains 10 or 12 transmembrane domains (fig. 3). The glyscosylation sites are located in a long external loop between transmembrane domains 1 and 2. The amino-terminal domain is sufficient to transport the ions in an amiloride-sensitive manner [27]. The C-terminal region features an adenosine triphosphate (ATP) binding site, two calmodulin binding sites and a calcineurin homolog protein (CHP) binding region that all confer Ca^{2+} sensitivity to NHE1. The C-terminal tail of the antiport also contains many phosphorylation sites and is thus involved in neurohormonal regulation of the ion-exchange activity [28].

The Na^+/H^+ antiport is regulated by cell volume [29, 30]. NHE-1 is stimulated by hypertonicity. Under similar conditions, NHE-2 activity is also increased. In cells incubated in hypotonic media, both isoforms are inhibited. In contrast, NHE-3 is markedly inhibited by hypertonic cell shrinking but is unaffected by hypotonicity [31]. A volume-sensitive domain has been mapped in NHE1 [32, 33]. The volume- or osmolarity-sensitive site(s) exist between the NH_2-terminal domain and residue 566 of NHE1 (fig. 3) [34]. A phosphorylation-independent mechanism likely underlies the volume regulation of the antiport [32]. This volume-sensitive site is therefore different from the site(s) postulated to mediate the stimulatory effects of calcium and growth factors.

The pH sensitivity of NHE1 is a complex mechanism. It is regulated by an autoinhibitory domain (AA 636–656) that prevents protonation of the pH sensor [35] and another domain that is more directly responsible for the maintenance of pH sensitivity (AA 567–635) [28]. Amino acids 515–595 in the C-terminal domain are particularly critical for the pH sensitivity of the antiport [36].

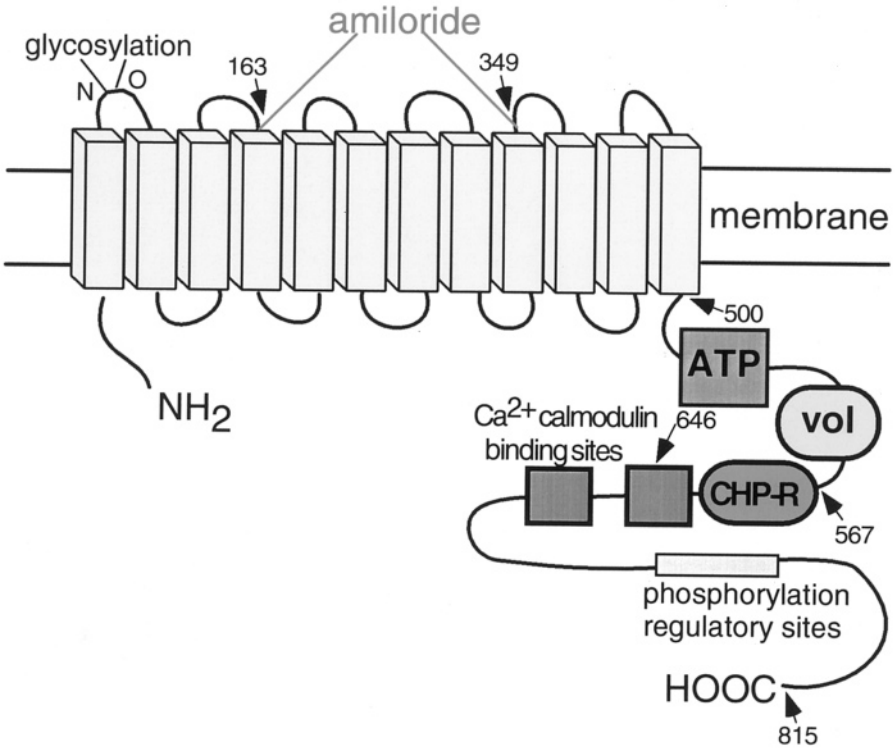

Figure 3. Structure of an Na^+-H^+ antiport: NHE1. vol, volume-regulatory domain; CHP-R, calcineurin homolog protein binding domain. Amino acid numbers are indicated by an arrow. (Modified from [42].)

Besides NHE1, NHE2, NHE3 and NHE4 [37–40] have been cloned. These isoforms have a more restricted tissue distribution. They are expressed in the gastrointestinal tract and in the kidney. NHE2 and NHE4 are also poorly expressed in brain, testis, lung, skeletal muscle and heart. In epithelial polarized cells, NHE1 was found mainly at the basolateral membrane. Like NHE1, NHE2, NHE3 and NHE4 are likely to display 12 transmembrane domains. NHE1 is inhibited by amiloride, and its derivatives such as ethylisopropylamiloride (EIPA) or dimethylamiloride (DMA). NHE3 is resistant to these drugs (EC_{50}: 100 μM for amiloride, 650 μM for HOE694), suggesting that the amino acid sequence that binds amiloride is not conserved in the NHE family. Indeed, a critical amino acid (Phe 167) was found to be responsible for the amiloride sensitivity of NHE1 [41]. While the amino-terminal domain is quite conserved, the C-terminal region features much less homology among the NHE isoforms. NHE2 and NHE3 lack the multiple phosphorylation sites of NHE1. NHE2 displays two proline-rich domains that bind SH3 domains [42]. The volume regulatory domain is missing in NHE3. NHE5 was found in nonepithelial tissus, including brain, spleen, testis and skeletal muscle [43]. It is amiloride-insensitive. NHE6 is expressed in numerous tissues with the highest messenger RNA (mRNA) level in brain and skeletal muscle. It may mediate mitochondrial Na^+/H^+ exchange [42].

Regulation of the Na^+/H^+ antiport by intracellular ATP

A drop in intracellular ATP dramatically decreases the activity of the Na-H antiport in several cell types. This effect was observed independently of the NHE isoform expressed (NHE1, NHE2 or NHE3) [31, 36], demonstrating that the ATP regulatory site is conserved among the members of the NHE family. The ATP dependence of NHE1 cannot only be explained by the requirement of phosphorylation, since mutation of all known phosphorylation sites [44] does not abolish the metabolic regulation of the antiport. The dependence of the antiport upon ATP is thus rather complex; the cytoskeleton is likely to participate in such a mechanism. A nondiffusible effector that binds ATP is probably required to activate the antiport in the presence of ATP [30].

Regulation of the Na^+/H^+ antiport by neurohormones and growth factors

It has been recognized for many years that, besides protons, the Na^+/H^+ antiport is regulated by external agonists including hormones and growth factors. Proton affinity or the maximal rate of ion exchange (V_{max}) of NHEs and thus the rate of ionic transport is increased when the cell is stimulated by neurohormones or growth factors. Phosphorylation of NHE proteins was first proposed to be responsible for activation of ion transport, because okadaic acid, a serine threonine phosphatase inhibitor, alone or together with growth factors induces activation of the antiport [45].

Protein kinase C (PKC) has been involved in such a regulation. Human but not rabbit NHE1 [46] displays a PKC consensus site (Ser 648). However, mutation of this amino acid does not affect phorbol ester- or thrombin-induced activation of the ionic exchange [28]. Direct phosphorylation of NHE1 by PKC is probably not required for activation of the antiport. Furthermore, PKC stimulation has various effects on the rate of Na^+/H^+ exchange. The phorbol ester PMA inhibits NHE3 activity but activates NHE1 and NHE2 expressed in PS120 NHE-deficient cells [47]. Growth factors and serum that also activate PKC stimulate the three NHE isoforms in PS120 cells [48]. It should be noted that NHE1 is mainly activated following a rise in proton affinity [49, 50], whereas the V_{max} of NHE2 and NHE3 is regulated by external stimuli [47, 48].

NHE1 does not feature protein kinase A (PKA) phosphorylation sites. The effect of PKA on NHE activity is quite controversial and depends upon the cell type and in turn upon the NHE isoform expressed. NHE activity can thus be either activated or inhibited by agents that increase intracellular cyclic adenosine monophosphate (cAMP). The regulation of NHE activity by PKA is even less simplistic. NHE3 is activated by treatment of cells with the β-adrenergic agonist isoproterenol but inhibited by forskolin stimulation that directly activates adenylyl cyclase [51]. Recently, Lefkowitz's group elucidated this apparent discrepancy. They showed that the occupancy of the β_2 adrenergic receptor with isoproterenol triggers the binding to the receptor of the NHE regulatory factor (NHERF), a factor that inhibits the Na^+-H^+ antiport in a PKA-dependent manner. NHERF contains two distinct protein interaction PSD95/Dlg/Zo-l (PDZ) domains: NHE-RF-PDZ1 and NHE-RF-PDZ2. NHERF binds to NHE3 through one of these PDZ domains. Binding of NHERF to the β_2-adrenergic receptor displaces NHERF from the antiport and thus relieves the inhibition of NHE3 [52]. A human homolog of NHERF was recently cloned. This homolog, the neurofibromatosis 2 tumor suppressor protein binds to merlin, an ERM (ezrin, radixin, moesin) family protein that makes the link with the cytoskeleton actin. The protein complex NHE-NF2 tumor suppressor ERM suggests a potential role of NHE3 in cytoskeleton rearrangement [53]. Further to the role of NHERF, it is interesting to briefly point out that a novel Na^+/H^+ exchange activity that requires Cl^- ions was recently found in crypt cells of rat distal

colon. This activity is fully inhibited by 0.5 mM DIDS, an anion-exchange inhibitor and by 0.1 mM 5-nitro-2-(3-phenylpropylamino)benzoic acid, a Cl^- channel blocker. A polyclonal antibody to the cystic fibrosis transmembrane conductance regulator (CFTR) partially inhibits Cl^--dependent proton gradient-driven Na^+ uptake, suggesting that the Cl^- dependence of Na^+/H^+ exchange involves a Cl^- channel that may be the CFTR and/or the outward rectifying Cl^- conductance [54]. It was recently reported that NHE-RF-PDZ1 binds to the CFTR C-terminus. This protein-protein interaction further suggests a potential regulatory role of NHE-RF CFTR function [55].

NHE1 displays two Ca^{2+}-calmodulin binding sites and a calcineurin homologous protein (CHP) binding domain that also binds Ca^{2+}. Binding of phosphorylated CHP inhibits the antiport [56]. A 24-kDa protein, which has not been identified, coimmunoprecipitates with NHE1 [57]. It is striking that 24 kDa is the size of CHP. This observation allows for speculation that it may indeed be CHP. 'In vivo', Ca^{2+} has been reported to have no effect [50], to decrease [58] or to increase the antiport activity [59]. Whether a Ca^2-calmodulin dependent kinase regulates the activity of NHE has not been yet proven. A direct effect of Ca^{2+} or calmodulin on NHE may also explain the stimulatory effect of Ca^2 on NHE activity [60, 61].

Pouyssegur's group proposed as early as 1991 [45] that a specific NHE1 kinase may be phosphorylating NHE1 following cell stimulation with growth factors. The NHE kinase would be downstream of MAPK (mitogen activated protein kinase), which integrates the signal from both PKC- and tyrosine kinase-dependent pathways. Using a dominant-negative form of p44 MAPK and inhibitors of MAPK kinase, Bianchini et al. found that the p42/p44 MAPKs play a predominant role in NHE1 regulation by neurohormones and growth factors [62]. Recently, Berk's group identified $p90^{rsk}$ as a potential NHE kinase. Both inhibition of p42/p44 MAPK and Ca^{2+} chelating agents prevent $p90^{rsk}$ activation, demonstrating that this NHE kinase is downstream of a MAPK signaling pathway. $p90^{rsk}$ may mediate the angiotensin II-induced activation of the Na^+/H^+ antiport in vascular smooth cells in hypertension [63, 64].

Regulation of NHE by G proteins

The G protein-mediated mode of regulation emerged a few years ago and has opened a new field of investigation. In 1994, the small oncogenic G protein Ras was proposed as a long-term regulator of NHE activity [65]. The same year, a constitutively activated form of $G_{\alpha 13}$, a member of the G_q family, expressed in HEK293 cells was reported to activate the Na^+/H^+ exchange mediated by NHE1 [66]. Later on, the same group found that $G_{\alpha 13}$ activates the antiport through independent stimulation of Cdc42 and RhoA. Cdc42 effect is mediated by MEKK1 (MAPK kinase kinase), whereas RhoA is not [67]. Furthermore, activation of NHE by RhoA is required for the formation of actinic stress fibers, a phenotype typical of RhoA activation [68]. This clearly indicates that Na-H antiport stimulation and thus change in pH_i is an event that occurs downstream of $G_{\alpha 13}$ and RhoA activation [69]. This signaling pathway that involves p160 Rho kinase regulates integrin-induced cell adhesion [70, 71]. In contrast, expression of a constitutively activated form of $G_{\alpha 12}$ in HEK293 and CCL19 fibroblasts inhibits NHE1 ion-exchange activity but stimulates NHE2 and NHE3 ion exchange [72]. Both $G_{\alpha 12}$ and $G_{\alpha 13}$ activate a MAPK-dependent pathway and induce cell transformation. This specific effect of $G_{\alpha 12}$ and $G_{\alpha 13}$ on NHE1 activity needs to be further investigated to understand the role of Na^+/H^+ activation in the cell transformation process.

The proteins of the AE family mediate Na^+-independent Cl^-/HCO_3^- exchange

Cl^-/HCO_3^- exchange has been extensively studied in erythrocytes. The Band3 protein that mediates erythroid Cl^-/HCO_3^- exchange is a glycoprotein that represents 30% of the membrane proteins in this cell type. Kopito and Lodish [13] cloned and sequenced the first gene encoding the 100-kDa Band3 protein and named it the Anion Exchanger 1 (AE1). Since then, two other members of a multigenic AE family have been found. The AEs are ubiquitously distributed in vertebrate tissues and are expressed very early during embryogenesis. As early as the two-cell stage, embryos are able to recover from an alkaline load by switching on the activity of a Cl^-/HCO_3^- exchanger [73, 74]. This is particularly important for the embryo that lives in a surrounding alkaline uterine environment [75].

In fact, the AE family comprises three genes, AE1, AE2 and AE3 [76], localized to separate chromosomes (17, 7 and 2, respectively) [77]. These genes encode three different proteins. AE1 is expressed in spleen and encodes the erythroid Band3 protein [78]. AE2 (130 to 180 kDa) is a housekeeping gene that is expressed in most tissues at least at the level of mRNA [79]. The AE2 protein is mainly found in stomach, kidney [80, 81] and brain (G. Bosman, personal communication). We also found AE2 immunoreactivity in whole heart but not in isolated cardiac cells, suggesting an expression restricted to vascular smooth muscle cells, fibroblasts and/or endothelial cells. AE3 is expressed strongly in brain as a 160-kDa protein [82] and in heart as a 120-kDa protein [77, 83–85].

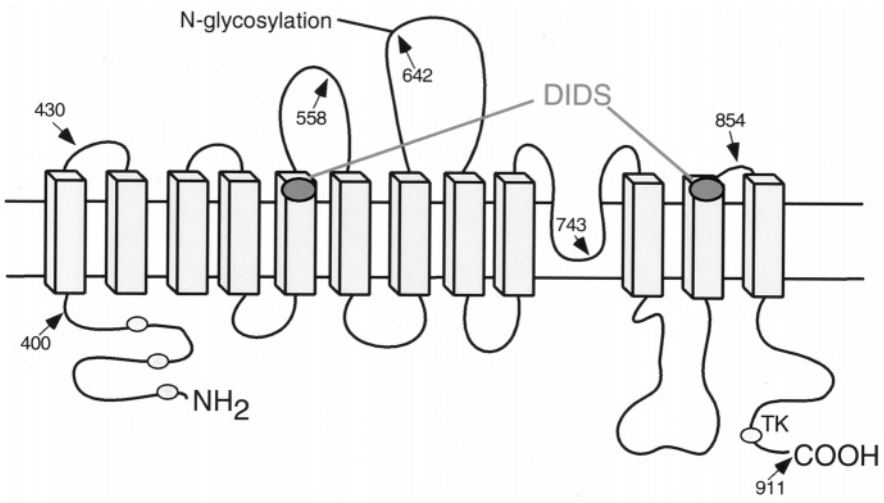

Figure 4. Structure of an Na^+-independent Cl^-/HCO_3^- exchanger: AE1. TK, tyrosine kinase phosphorylation site. Amino acid numbers are indicated by an arrow. (Modified from [86].)

The three AE isoforms share 65% amino acid sequence homology in the membrane-associated transport domains, diverging to a greater extent in the N-terminal, cytoplasmic portion [76]. The AE proteins exhibit 12 transmembrane domains (fig. 4). The most recent proposed topologies of the protein [86, 87] show a large extracellular loop between domains 7 and 8 that is N-glycosylated. The AE1 and AE3 proteins are strongly inhibited by 4,4′-diisothiocyanatodihydrostilbene-2,2′-disulphonate DIDS (EC_{50}: 1–10 μM), whereas AE2 is poorly sensitive to the inhibitor (EC_{50}: 1 mM) [88]. DIDS binding sites have been localized to lysines 539 and 851 in the 5th and 11th transmembrane domain of AE1 [86, 87]. The sequence KL(X)K (where X = I, V or Y) can be proposed as a consensus sequence of amino acids for DIDS binding from the alignment of AE1, AE2 and AE3 as well as from the knowledge of DIDS reactive lysines.

Further sequence diversity in the cytoplasmic domain results from alternative splicing of each gene product. Two isoforms of AE1, eAE1 and kAE1, have been cloned, and represent the major isoforms present in erythrocytes and kidney, respectively. The kAE1 isoform represents a truncation of the eAE1 protein starting at Met-79 of eAE1 [89, 90] and thus encoding an 80-kDa polypeptide. We have also identified by polymerase chain reaction (PCR) mapping a new AE1 spliceoform in heart that encodes an 80–90 kDa protein different from kAE1 [91]. AE2 transcription can be initiated at three different points, generating alternatively spliced mRNAs and three proteins (AE2a, AE2b and AE2c) N-terminally truncated at different points within the first 200 amino acids, and migrating in SDS-PAGE with apparent molecular masses of 115 to 180 kDa [79]. AE3 transcripts comprise two isoforms present at highest concentrations in brain (bAE3) [82] or in cardiac tissue (cAE3). The 269 N-terminal amino acids of bAE3 are replaced by 73 alternate residues in cAE3. However, these isoforms are not strictly specific to tissues; cardiac cells express both cAE3 and bAE3 [92], and brain expresses both AE3 variants [91]. The specific expression of AE isoforms and spliceoform proteins in different tissues is still poorly documented. We have shown that a single cell type such as a cardiomyocyte can express as many as four AE proteins [91].

The most striking characteristic of the AE family is the diversity of functions fulfilled by its members. Indeed, besides pH_i regulation, the AEs modulate chloride and bicarbonate homeostasis in tissues such as stomach and kidney [76]. AE1 also contributes to the flexibility of the erythrocyte cytoskeleton and in turn to cell shape [93]. AE1 regulates glycolysis in erythrocytes by binding and inhibiting aldolase, glyceraldehyde 3-phosphate dehydrogenase and phosphofructokinase [94]. Moreover, AE1 generates a senescent antigen, a marker for cell removal by the immune system [95]. In response to swelling, cells recover their initial volume by releasing intracellular ions or small molecules such as amino acids via volume-regulated pathways. Like many other ion channels and transporters [96], AE1 as well as AE2 participate in the regulation of cell volume [97]. For example, trout AE1 (tAE1) has been shown to work as a bifunctional protein with both anion exchange and Cl^-/taurine channel functions [97].

Although slightly beyond the scope of this review, it is interesting to briefly point out that the association of some AE functions with particular domains in the protein emphasizes the power of alternative splicing in increasing the diversity of AE functions. For example, AE2 and AE3 contain an insertion in the 'Z-loop' (the extracellular peptide loop between membrane spans 5 and 6) that confers cell volume regulatory ability to the AE protein and that is absent from AE1 [97]. Only the eAE1 protein, however, contains regions in its cytoplasmic portion responsible for binding ankyrin or aldolase, which is absent from the kAE1 isoform [98, 99]. Alternatively, kanadaptin, a recently identified protein binds to kAE1 but not eAE1 [100]. Similarly, it has been postulated that the addition of 17 N-terminal residues in AE2a is responsible for a switch in targeting of the protein, from basal membranes in ileal enterocytes to apical membranes in gastric parietal cells [79]. A better knowledge of the structure of the AE proteins is expected to provide a better understanding of the function and regulation of these proteins.

pH dependence of the AE proteins

The pH dependence of AE1 follows a characteristic pattern. The anion exchange rate increases up to pH 7, then reached a plateau in the pH range 7–11 and shows a sharp decrease [101]. In contrast, AE2 and AE3 expressed in HEK293 cells or in *Xenopus* oocytes exhibit a pH regulation within a more physiological range of pH (pH 7–9) [88, 102]. Using chimeric and truncated AE expressed in oocytes, Zhang et al. [102] proposed that the pH sensor resides in a transmembrane domain of AE2 whose affinity for protons is regulated by a domain located between amino acids 99 and 510 in the N-terminal domain of AE2. Using site-directed mutagenesis, Müller-Berger et al. found that a hydrogen bond between histidine 752 and glutamate 699 is essential in the pH dependence of AE1 below pH 8.3 [103, 104]. The role of glutamate 699 in the sensitivity of AE to protonation was confirmed for both AE1 and AE2 [105].

Neurohormonal regulation of the AE proteins

Many agonists activate the Cl^-/HCO_3^- exchanger in various cell types. Ganz et al. [106] observed in mesangial cells that vasopressin activates Cl^-/HCO_3^- exchange. Prostaglandin facilitates Cl^-/HCO_3^- exchange in amphibian oxynticopeptic cells [107]. The cAMP-dependent pathway has often been involved in the regulation of AE activity. A cell-permeant cAMP analog dibutyryl cAMP and forskolin activate the Cl^-/HCO_3^- exchanger in renal epithelial cells [108]. In hepatocytes, glucagon that increases intracellular cAMP accelerates anionic exchange [109]. We also observed in cardiac cells that β-adrenergic stimulation and in turn an increase in cAMP accelerate the rate of Cl^-/HCO_3^- exchange [110].

Besides PKA, other signal transduction pathways regulate the activity of AE. We found in 1991 that the purinergic agonist stimulates the Cl^-/HCO_3^- exchanger in cardiac cells. The DIDS-sensitive stimulation of the Cl^-/HCO_3^- exchanger leads to a cytosolic acidification up to 0.4 unit pH [111]. The purinergic agonist ATP has been more recently reported to activate the Cl^-/HCO_3^- exchanger in osteoclasts [112] and in neurons [113]. These data prompted us to investigate which isoform mediates the purinergic activation of the Cl^-/HCO_3^- exchanger and the signal transduction pathway that underlies the activation of the anionic exchanger.

The purinergic activation of AE is observed in cells in which AE3 expression was blocked by antisense oligonucleotide but not in cells microinjected with neutralizing anti-AE1 [92] antibodies. Erythroid AE1 features several potential tyrosine phosphorylation sites [114, 115] and has been shown to be an in vitro substrate for several tyrosine kinases, including Src, Abl, the EGF receptor, Syk [116] and ZAP-70 [117]. ATP stimulation of cardiomyocytes also induces tyrosine phosphorylation of AE1. The purinergic agonist increases the activity of the tyrosine kinases $pp60^{c\text{-}src}$ (Src) and $pp59^{Fyn}$ (Fyn) and induces association of both Fyn and FAK (focal adhesion kinase) with AE1. Inhibition of Src-family kinases in vivo by genistein, herbimycin A or ST638 prevents purinergic activation of AE1. Microinjection of either anti-Cst.1 antibody or recombinant CSK, both of which prevent activation of Src-family kinases, significantly decreases ATP-induced activation of AE. Microinjection of an anti-FAK antibody as well as expression in cardiomyocytes of Phe397 FAK dominant-negative mutant also prevents purinergic activation of AE. We have thus proposed that ATP-induced phosphorylation of FAK leads to recruitment of Fyn close to the AE1 protein and that phosphorylation of the AE protein by Fyn accelerates its rate of ionic transport [92]. Tyrosine kinases turn out to be major regulators of AE activity in numerous cell types.

The role of PKC in regulating AE proteins has not been extensively studied. It is still a much more controversial issue than the participation of tyrosine kinase in AE regulation. In vero cells, the phorbol ester TPA activates the Cl^-/HCO_3^- exchanger [118]. Although angiotensin II-induced increase in AE activity was reported to be mediated by PKC in cardiac papillary muscle [119], we did not observe any effects of phorbol ester on the activity of the anionic exchanger in isolated ventricular cells (M. Puceat, unpublished data).

Na^+-dependent bicarbonate transporters

Several Na^+-HCO_3^- transporters have been identified in most cell types. They mediate both a cell influx and a cell efflux of HCO_3^- ions. They are thus either alkalinizing or acidifying transporters. In 1990, Liu et al. [120] identified in embryonic chicken heart cells an Na^+-dependent Cl^-/HCO_3^- exchanger. This electroneutral exchanger extrudes Cl^- and H^+ in exchange for Na^+ and HCO_3^- ions and thus regulates pH after an acid load. Such an exchanger has also been described in the kidney [121].

Besides this exchanger, Na^+-HCO_3^- cotransporters have been shown to play an important role in pH_i regulation of various tissues [121–125]. These transporters belong to a broad family of Na-bicarbonate cotransporters (NBCs). Functional studies support the existence of NBCs that work in both directions; Na^+ and HCO_3^- ions are extruded from the proximal tubule of kidney in which the NBCs play a major role in the filtering of HCO_3^- ions. Using an expression cloning strategy, the kidney NBC was the first NBC cloned from a kidney salamander complementary DNA (cDNA) library and named NBC3 because of its stochiometry [126]. This DIDS-sensitive cotransport carries 1 Na^+ and 3 HCO_3^- ions out of the cell and is thus the most electrogenic transporter known. The sequence of NBC3 predicts a protein of 1035 amino acids featuring 10 transmembrane domains (fig. 5). It is highly expressed in kidney, and the 4.2-kb mRNA transcript was also found in bladder, intestine, eye and brain. The brain and eye transcripts suggest that it may be the cotransporter found in retinal glial cells [127–129]. Other proteins related to NBC with stochiometry Na^+:HCO_3^- 1:2 are likely to be expressed in glial [130], hepatic [131], colon [124] and pancreatic cells [132].

Romero et al. recently cloned from a rat cortex kidney cDNA library a mammalian homolog of NBC3 named rNBC [133]. rNBC encodes a protein of 1035 amino acids with a predicted molecular mass of 116 kDa. This N-glycosylated protein mediates an efflux of both Na^+ and HCO_3^- ions. It has one PKA and tyrosine kinase and several PKC and casein kinase II phosphorylation sites, suggesting numerous ways of regulation. Like NBC3, rNBC has a poor homology with the AE family (30% identity in the amino acid sequence). Northern blot analysis of RNA from various tissues reveals that the 7.5-kb rNBC transcript is strongly detected in kidney, brain and liver; a weak signal appears in lung and heart.

A novel member of the NBC family that carries Na^+ and HCO_3^- ions into the cell was cloned from a human kidney cDNA library called NBC1 [134]. The electrogenic transporter is unable to work in the efflux mode. The 7.6-kb cDNA predicts a 116-kDa protein with 1035 amino acids. NBC1 has 29% homology with AE3. Expressed in HEK 293 cells, NBC1 facilitates the recovery of pH_i from an acid load in the presence of amiloride. The Na^+-HCO_3^- cotransport can function as an Na^+-OH^- cotransport at high external pH; the physiological significance of this cotransport may be found in the

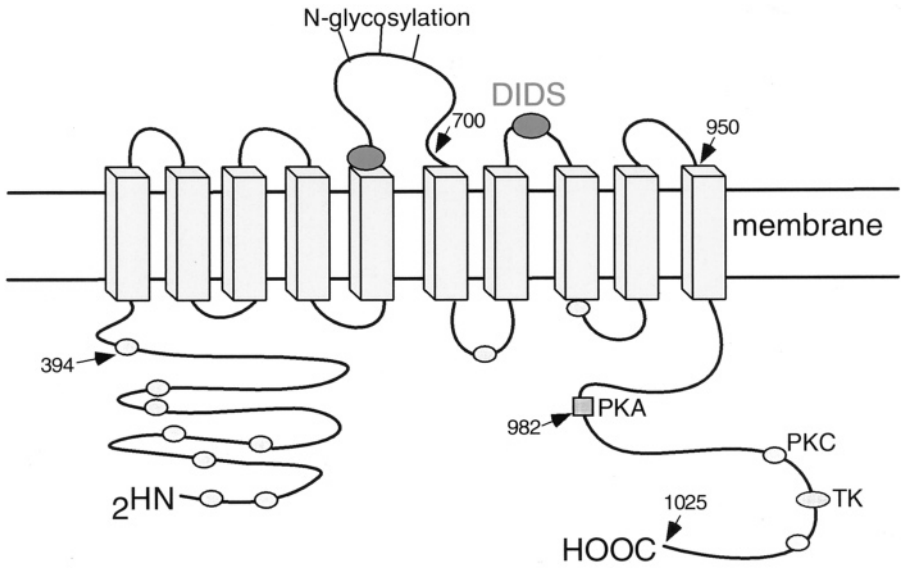

Figure 5. Structure of an Na^+-HCO_3^- cotransporter: NBC3. TK, tyrosine kinase phosphorylation site; PKC, protein kinase C phosphorylation site; PKA, protein kinase A phosphorylation site. (Modified from [137].)

colon. NBC1 is inhibited by 300 μM DIDS and 200 μM harmaline [135].
A rat kidney NBC1 cDNA with a high homology with human NBC1 also strongly expressed in brain and colon was recently reported by the same group [136] but turns out to be identical to rNBC [137].
A pancreatic NBC 7.7-kb cDNA was recently cloned by searching the human expressed sequence tag database [138]. The gene encodes a 1079-amino acid polypeptide that mediates an uptake of Na^+ and HCO_3^- ions when expressed in oocytes. The gene is localized to chromosome 4, position 4q21. The mRNA was predominantly found in both acini and ductal cells of pancreas with less expression in kidney, brain, liver, prostate, colon, stomach, thyroid and spinal chord.
A major difference in regard to the NBCs between the kidney and the other tissues is the direction of the ionic transport. Both ionic composition of intracellular and extracellular media and cell membrane potential may explain this difference. Alternatively, different isoforms of NBC may mediate influx or efflux of Na^+ together with HCO_3^- ions. In this regard, a functional Na^+-HCO_3^- cotransporter that transports the ions into the cell was also found in cardiomyocytes. Lagadic Gossmann et al. [139] did not find any evidence about electrogenicity of this cotransport and thus proposed a stochiometry of 1 Na^+:1 HCO_3^-. An electrogenic inward-directed Na^+- HCO_3^- cotransport activated by membrane depolarization was observed in heart [140, 141].

Neurohormonal regulation of the NBC proteins

As expected from the presence in the NBC proteins of many protein kinases consensus sites, the activity of the Na^+-HCO_3^- cotransporters is regulated by neurohormones. Arginine vasopressin activates the cotransport in mesangial cells [106]; the signaling pathways underlying this effect have not been sought. Functional studies have shown in kidney an inhibition of Na^+-HCO_3^- cotransport by PKC and PKA [142]. A β-adrenergic agonist that increases intracellular cAMP also decreases the activity of the Na^+-HCO_3^--cotransport in cardiac cells [143]. Terzic et al. found that both an α_1-adrenergic agonist and the purinergic agonist that activate PKC stimulate an amiloride-insensitive and bicarbonate-dependent alkalinizing transporter in rat cardiac cells [144]. The role of PKC or other kinases has not been further investigated. In the same cell type, Kohout and Rogers [145] observed a stimulation of the Na^+-HCO_3^- cotransport by angiotensin II. This effect was mimicked by arachidonic acid. Staurosporine, a PKC inhibitor, prevented both arachidonic acid and angiotensin II stimulatory effect on the Na^+-HCO_3^- cotransport. In contrast to data obtained in kidney, PKC activates the cotransport in cardiac cells. The cardiac Na^+-HCO_3^- cotransporter has not been cloned. An isoform different from NBC1 or NBC3 may explain a differential protein kinase regulation. In contrast to kidney cells, cardiomyocytes have an unstable membrane potential. This may also be of importance for regulation of an electrogenic Na^+-HCO_3^- cotransporter.

pH regulatory ion transporters and pathophysiologies

Numerous pathologies have been associated with mutation or absence of expression of the Band3 protein [146–150]. A few of them lead to changes in anionic exchange activity. Inaba et al. [151] reported a defective Cl^-/HCO_3^- exchange in a bovine anemia associated with a lack of erythroid Band3. A familial distal renal tubular acidosis associated with a mutation of the kidney AE1 protein has recently been well documented. The mutation of Arginine 589 located in the C-terminal domain of kAE1 results in systemic metabolic acidosis due to failure of appropriate proton secretion in the distal nephron. Arginine 589 is replaced by a histidine or a cysteine. A mutation of Serine 613 to a phenylalanine was also found in two families [152]. The erythrocytes of patients carrying the arginine mutation show a 20% decrease in ion exchange. Expression of mutated kAE1 in oocytes also results in 20% to 50% decrease in Cl^-/HCO_3^- exchange [153]. The Serine mutation increases sulphate but not iodide transport of red cells [152].
NHE proteins have also been involved in the pathogenesis of diseases. Microvillus inclusion disease is a congenital disorder characterized by secretory diarrhea. The patients with this disease have defects in apical but not basolateral duodenal membrane transport systems. This defect is at the origin of the disease [154]. That NHE is an important mediator of ischemic-reperfusion injury of the heart is more and more evident. During ischemia, the cardiac cell acidifies, activating in turn the Na^+/H^+ antiport. This leads to a cellular Na^+ load. During reperfusion, the Na^+/Ca^{2+} exchanger is turned on, inducing Ca^{2+} overload and cell damage [155]. Inhibitors of NHE activity turn out to be potential beneficial drugs for the prevention of ischemic-reperfusion injury [156].
Cox et al. [157] found a spontaneous mutation of the NHE1 protein in mouse. These mice that are NHE1-deficient show a neurological syndrome that includes ataxia and a unique epilepsy phenotype (slow wave epilepsy). Selective neuronal death was observed in brain of the mutant. The lack of NHE1 was not compensated for by expression of another NHE isoform. Surprisingly, besides brain, the function other organs

also missing NHE1 appear not to be affected. We have investigated the expression of the AE isoforms in the brain and heart of the mutant mice but did not find any change when compared with wild-type mice. We are in the process of looking at pH regulation in cardiac cells isolated from these mice (M. Pucéat, unpublished data).

Conclusions

In the last decade, many pH regulatory ion transporters have been cloned and sequenced. The structure and topology of these ion exchangers, cotransporters or antiports has yielded much information about the pH regulatory role and neurohormonal regulation of these proteins. More isoforms of NHE and AE proteins remains to be discovered. Undoubtedly, many NBC members are still unkown. Substantial progress is expected to be made in the near future to identify all these membrane proteins.

The intracellular localization of NHE, AE and NBC proteins is still poorly documented. NHE1 expressed in PS120 cells colocalized with vinculin, actin and F-actin, components of the focal adhesion when the cells were attached [158]. AE1 protein is linked to ankyrin in the red cell and is associated with costameres in cardiac cells, a structure similar to focal adhesions [84]. Cardiac AE1 is coimmunoprecipitated with FAK [92]. Besides the plasma membrane, NHE, AE and NBC proteins are likely to be localized to the nuclear membrane, Golgi apparatus or mitochondria. Using GFP-fusion proteins, we should learn more in the future about the sorting and targeting of pH regulatory ion transporters. The use of pH-sensitive targeted GFP proteins should provide us with new concepts of intracellular pH regulation that depend not only on exchange of H^+ across the plasma membrane but also across the membrane of organelles. Similarly to Ca^{2+} microdomains [159], a concept of H^+ microenvironments close to the pH regulatory ion exchanger or around the intracellular compartments needs to be elaborated. Finally, as briefly discussed above, pathologies associated with NHE, AE or NBC mutations must be sought.

1 Busa W. B. and Nuccitelli R. (1984) Metabolic regulation via intracellular pH. Am. J. Physiol. **246:** R409–438
2 Isfort R. J., Cody D. B., Asquith T. N., Ridder G. M., Stuard S. B. and LeBoeuf R. A. (1993) Induction of protein phosphorylation, protein synthesis, immediate- early-gene expression and cellular proliferation by intracellular pH modulation. Implications for the role of hydrogen ions in signal transduction. Eur. J. Biochem. **213:** 349–357
3 Orchard C. H. and Kentish J. C. (1990) Effects of changes of pH on the contractile function of cardiac muscle. Am. J. Physiol. **258:** C967–981
4 Tominaga T., Ishizaki T., Narumiya S. and Barber D. L. (1998) Na-H exchange acts downstream of RhoA to regulate integrin-induced cell adhesion and spreading. EMBO J. **17:** 4712–4722
5 McConkey D. J. and Orrenius S. (1996) Signal transduction pathways in apoptosis. Stem Cells **14:** 619–631
6 Gottlieb R. A., Gruol D. L., Zhu J. Y. and Engler R. L. (1996) Preconditioning rabbit cardiomyocytes: role of pH, vacuolar proton ATPase and apoptosis. J. Clin. Invest. **97:** 2391–2398
7 Needham J. and Needham D. M. (1926) The hydrogen ion concentration and the oxidation-reductionpotential of the cell interior: a microinjection study. Proc. R. Soc. London Ser. B **98:** 259–286
8 Al-Awqati Q. (1986) Proton-translocating ATPases. Annu. Rev. Cell Biol. **2:** 179–199
9 DeCoursey T. E. (1998) Four varieties of voltage-gated proton channels. Front. Biosci. **3:** d477–482
10 Murer H., Hopfer U. and Kinne R. (1976) Sodium/proton antiport in brush-border-membrane vesicles isolated from rat small intestine and kidney. Biochem. J. **154:** 597–604
11 Cremaschi D., Henin S. and Meyer G. (1979) Stimulation by HCO_3^- of Na^+ transport in rabbit gallbladder. J. Membr. Biol. **47:** 145–170
12 Russell J. M. and Boron W. F. (1976) Role of chloride transport in regulation of intracellular pH. Nature **264:** 73–74
13 Kopito R. R. and Lodish H. F. (1985) Primary structure and transmembrane orientation of the murine anion exchange protein. Nature **316:** 234–238
14 Leem C. H. and Vaughan-Jones R. D. (1998) Sarcolemmal mechanisms for pHi recovery from alkalosis in the guinea-pig ventricular myocyte. J. Physiol. (Lond.) **509:** 487–496
15 Rosenberg S. O., Fadil T. and Schuster V. L. (1993) A basolateral lactate/H^+ co-transporter in Madin-Darby Canine Kidney (MDCK) cells. Biochem. J. **289:** 263–268
16 Wang X., Levi A. J. and Halestrap A. P. (1994) Kinetics of the sarcolemmal lactate carrier in single heart cells using BCECF to measure pHi. Am. J. Physiol. **267:** H1759–1769
17 Roos A. and Boron W. F. (1981) Intracellular pH. Physiol. Rev. **61:** 296–434
18 Bassnett S., Reinisch L. and Beebe D. C. (1990) Intracellular pH measurement using single excitation-dual emission fluorescence ratios. Am. J. Physiol. **258:** C171–178
19 Mordon S., Devoisselle J. M. and Soulie S. (1995) Fluorescence spectroscopy of pH in vivo using a dual-emission fluorophore (C-SNAFL-1). J. Photochem. Photobiol. B. **28:** 19–23
20 Zhou Y., Marcus E. M., Haugland R. P. and Opas M. (1995) Use of a new fluorescent probe, seminaphthofluorescein-calcein, for determination of intracellular pH by simultaneous dual-emission imaging laser scanning confocal microscopy. J. Cell Physiol. **164:** 9–16
21 Llopis J., McCaffery J. M., Miyawaki A., Farquhar M. G. and Tsien R. Y. (1998) Measurement of cytosolic, mitochondrial, and Golgi pH in single living cells with green fluorescent proteins. Proc. Natl. Acad. Sci. USA **95:** 6803–6808
22 Counillon L., Scholz W., Lang H. J. and Pouyssegur J. (1993) Pharmacological characterization of stably transfected Na^+/H^+ antiporter isoforms using amiloride analogs and a new inhibitor exhibiting anti-ischemic properties. Mol. Pharmacol. **44:** 1041–1045
23 Sardet C., Franchi A. and Pouyssegur J. (1989) Molecular cloning, primary structure, and expression of the human growth factor-activatable Na^+/H^+ antiporter. Cell **56:** 271–280
24 Counillon L., Pouyssegur J. and Reithmeier R. A. (1994) The Na^+/H^+ exchanger NHE-1 possesses N- and O-linked glycosylation restricted to the first N-terminal extracellular domain. Biochemistry **33:** 10463–10469
25 Fafournoux P., Noel J. and Pouyssegur J. (1994) Evidence that Na^+/H^+ exchanger isoforms NHE1 and NHE3 exist as stable dimers in membranes with a high degree of specificity for homodimers. J. Biol. Chem. **269:** 2589–2596
26 Haworth R. S., Frohlich O. and Fliegel L. (1993) Multiple carbohydrate moieties on the Na^+/H^+ exchanger. Biochem. J. **289:** 637–640

27 Wakabayashi S., Fafournoux P., Sardet C. and Pouyssegur J. (1992) The Na^+/H^+ antiporter cytoplasmic domain mediates growth factor signals and controls 'H(+)-sensing'. Proc. Natl. Acad. Sci. USA **89:** 2424–2428

28 Wakabayashi S., Bertrand B., Shigekawa M., Fafournoux P. and Pouyssegur J. (1994) Growth factor activation and 'H(+)-sensing' of the Na^+/H^+ exchanger isoform 1 (NHE1). Evidence for an additional mechanism not requiring direct phosphorylation. J. Biol. Chem. **269:** 5583–5588

29 Demaurex N. and Grinstein S. (1994) Na^+/H^+ antiport: modulation by ATP and role in cell volume regulation. J. Exp. Biol. **196:** 389–404

30 Demaurex N., Romanek R. R., Orlowski J. and Grinstein S. (1997) ATP dependence of Na^+/H^+ exchange. Nucleotide specificity and assessment of the role of phospholipids. J. Gen. Physiol. **109:** 117–128

31 Kapus A., Grinstein S., Wasan S., Kandasamy R. and Orlowski J. (1994) Functional characterization of three isoforms of the Na^+/H^+ exchanger stably expressed in Chinese hamster ovary cells. ATP dependence, osmotic sensitivity and role in cell proliferation. J. Biol. Chem. **269:** 23544–23552

32 Grinstein S., Woodside M., Sardet C., Pouyssegur J. and Rotin D. (1992) Activation of the Na^+/H^+ antiporter during cell volume regulation. Evidence for a phosphorylation-independent mechanism. J. Biol. Chem. **267:** 23823–23828

33 Nath S. K., Hang C. Y., Levine S. A., Yun C. H., Montrose M. H., Donowitz M. et al. (1996) Hyperosmolarity inhibits the Na^+/H^+ exchanger isoforms NHE2 and NHE3: an effect opposite to that on NHE1. Am. J. Physiol. **270:** G431–441

34 Bianchini L., Kapus A., Lukacs G., Waban S., Wakabayashi S., Pouyssegur J. et al. (1995) Responsiveness of mutants of NHE1 isoform of Na^+/H^+ antiport to osmotic stress. Am. J. Physiol. **269:** C998–1007

35 Wakabayashi S., Ikeda T., Iwamoto T., Pouyssegur J. and Shigekawa M. (1997) Calmodulin-binding autoinhibitory domain controls 'pH-sensing' in the Na^+/H^+ exchanger NHE1 through sequence-specific interaction. Biochemistry **36:** 12854–12861

36 Wakabayashi S., Shigekawa M. and Pouyssegur J. (1997) Molecular physiology of vertebrate Na^+/H^+ exchangers. Physiol. Rev. **77:** 51–74

37 Tse C. M., Brant S. R., Walker M. S., Pouyssegur J. and Donowitz M. (1992) Cloning and sequencing of a rabbit cDNA encoding an intestinal and kidney-specific Na^+/H^+ exchanger isoform (NHE-3). J. Biol. Chem. **267:** 9340–9346

38 Orlowski J., Kandasamy R. A. and Shull G. E. (1992) Molecular cloning of putative members of the Na/H exchanger gene family. cDNA cloning, deduced amino acid sequence and mRNA tissue expression of the rat Na/H exchanger NHE-1 and two structurally related proteins. J. Biol. Chem. **267:** 9331–9339

39 Wang Z., Orlowski J. and Shull G. E. (1993) Primary structure and functional expression of a novel gastrointestinal isoform of the rat Na/H exchanger. J. Biol. Chem. **268:** 11925–11928

40 Collins J. F., Honda T., Knobel S., Bulus N. M., Conary J., DuBois R. et al. (1993) Molecular cloning, sequencing, tissue distribution, and functional expression of a Na^+/H^+ exchanger (NHE-2). Proc. Natl. Acad. Sci. USA **90:** 3938–3942

41 Counillon L., Franchi A. and Pouyssegur J. (1993) A point mutation of the Na^+/H^+ exchanger gene (NHE1) and amplification of the mutated allele confer amiloride resistance upon chronic acidosis. Proc. Natl. Acad. Sci. USA **90:** 4508–4512

42 Orlowski J. and Grinstein S. (1997) Na^+/H^+ exchangers of mammalian cells. J. Biol. Chem. **272:** 22373–22376

43 Klanke C. A. et al. (1995) Molecular cloning and physical and genetic mapping of a novel human Na^+/H^+ exchanger (NHE5/SLC9A5) to chromosome 16q22.1. Genomics **25:** 615–622

44 Goss G. G., Woodside M., Wakabayashi S., Pouyssegur J., Waddell T., Downey G. P. et al. (1994) ATP dependence of NHE-1, the ubiquitous isoform of the Na^+/H^+ antiporter. Analysis of phosphorylation and subcellular localization. J. Biol. Chem. **269:** 8741–8748

45 Sardet C., Fafournoux P. and Pouyssegur J. (1991) Alpha-thrombin, epidermal growth factor, and okadaic acid activate the Na^+/H^+ exchanger, NHE-1, by phosphorylating a set of common sites. J. Biol. Chem. **266:** 19166–19171

46 Fliegel L., Sardet C., Pouyssegur J. and Barr A. (1991) Identification of the protein and cDNA of the cardiac Na^+/H^+ exchanger. FEBS Lett. **279:** 25–29

47 Tse C. M., Levine S. A., Yun C. H., Brant S. R., Pouyssegur J., Montrose M. H. et al. (1993) Functional characteristics of a cloned epithelial Na^+/H^+ exchanger (NHE3): resistance to amiloride and inhibition by protein kinase C. Proc. Natl. Acad. Sci. USA **90:** 9110–9114

48 Tse C. M., Levine S. A., Yun C. H., Brant S. R., Pouyssegur J., Montrose M. H. and Donowitz M. (1993) Functional characteristics of a cloned epithelial Na^+/H^+ exchanger (NHE3): resistance to amiloride and inhibition by protein kinase C. Proc. Natl. Acad. Sci. USA **90:** 9110–9114

49 Paris S. and Pouyssegur J. (1984) Growth factors activate the Na^+/H^+ antiporter in quiescent fibroblasts by increasing its affinity for intracellular H^+. J. Biol. Chem. **259:** 10989–10994

50 Puceat M., Clement-Chomienne O., Terzic A. and Vassort G. (1993) Alpha 1-adrenoceptor and purinoceptor agonists modulate Na-H antiport in single cardiac cells. Am. J. Physiol. **264:** H310–319

51 Noel J. and Pouyssegur J. (1995) Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na^+/H^+ exchanger isoforms. Am. J. Physiol. **268:** C283–296

52 Hall R. A., Premont R. T., Chow C. W., Bitzer J. T., Pitcher J. A., Claing A. et al. (1998) The beta2-adrenergic receptor interacts with the Na^+/H^+-exchanger regulatory factor to control Na^+/H^+ exchange. Nature **392:** 626–630

53 Murthy A., Gonzalezagosti C., Cordero E., Pinney D., Candia C., Solomon F. et al. (1998) NHE-RF, a regulatory cofactor for Na^+-H^+ exchange is a common interactor for merlin and ERM (MERM) proteins. J. Biol. Chem. **273:** 1273–1276

54 Rajendran V. M., Geibel J. and Binder H. J. (1999) Role of Cl channels in Cl-dependent Na/H exchange. Am. J. Physiol. **276:** G73–G78

55 Wang S., Raab R. W., Schatz P. J., Guggino W. B. and Li M. (1998) Peptide binding consensus of the NHE-RF-PDZ1 domain matches the C-terminal sequence of cystic fibrosis transmembrane conductance regulator (CFTR). FEBS Lett. **427:** 103–108

56 Lin X. and Barber D. L. (1996) A calcineurin homologous protein inhibits GTPase-stimulated Na-H exchange. Proc. Natl. Acad. Sci. USA **93:** 12631–12636

57 Goss G., Orlowski J. and Grinstein S. (1996) Coimmunoprecipitation of a 24-kDa protein with NHE1, the ubiquitous isoform of the Na^+/H^+ exchanger. Am. J. Physiol. **270:** C1493–1502

58 Levine S. A., Nath S. K., Yun C. H., Yip J. W., Montrose M., Donowitz M. et al. (1995) Separate C-terminal domains of the epithelial specific brush border Na^+/H^+ exchanger isoform NHE3 are involved in stimulation and inhibition by protein kinases/growth factors. J. Biol. Chem. **270:** 13716–13725

59 Gupta A., Schwiening C. J. and Boron W. F. (1994) Effects of CGRP, forskolin, PMA, and ionomycin on pHi dependence of Na-H exchange in UMR-106 cells. Am. J. Physiol. **266:** C1088–1092

60 Bertrand B., Wakabayashi S., Ikeda T., Pouyssegur J. and Shigekawa M. (1994) The Na^+/H^+ exchanger isoform 1 (NHE1) is a novel member of the calmodulin-binding proteins. Identification and characterization of calmodulin-binding sites. J. Biol. Chem. **269:** 13703–13709

61 Wakabayashi S., Bertrand B., Ikeda T., Pouyssegur J. and Shigekawa M. (1994) Mutation of calmodulin-binding site renders the Na^+/H^+ exchanger (NHE1) highly H(+)-sensitive and Ca^{2+} regulation-defective. J. Biol. Chem. **269:** 13710–13715

62 Bianchini L. L., Allemain G. and Pouyssegur J. (1997) The p42/p44 mitogen-activated protein kinase cascade is determinant in mediating activation of the Na^+/H^+ exchanger (NHE1 isoform) in response to growth factors. J. Biol. Chem. **272:** 271–279

63 Takahashi E., Abe J. and Berk B. C. (1997) Angiotensin II stimulates p90rsk in vascular smooth muscle cells. A potential Na(+)-H^+ exchanger kinase. Circ. Res. **81:** 268–273

64 Phan V. N., Kusuhara M., Lucchesi P. A. and Berk B. C. (1997) A 90-kD Na(+)-H^+ exchanger kinase has increased activity in spontaneously hypertensive rat vascular smooth muscle cells. Hypertension **29:** 1265–1272

65 Kaplan D. L. and Boron W. F. (1994) Long-term expression of c-H-ras stimulates Na-H and Na(+)-dependent Cl-HCO_3 exchange in NIH-3T3 fibroblasts. J. Biol. Chem. **269:** 4116–4124

66 Voyno-Yasenetskaya T., Conklin B. R., Gilbert R. L., Hooley R., Bourne H. R. and Barber D. L. (1994) G alpha 13 stimulates Na-H exchange. J. Biol. Chem. **269:** 4721–4724

67 Hooley R., Yu C. Y., Symons M. and Barber D. L. (1996) G alpha 13 stimulates Na^+-H^+ exchange through distinct Cdc42-dependent and RhoA-dependent pathways. J. Biol. Chem. **271:** 6152–6158

68 Nobes C. and Hall A. (1994) Regulation and function of the Rho subfamily of small GTPases. Curr. Opin. Genet. Dev. **4:** 77–81

69 Vexler Z. S., Symons M. and Barber D. L. (1996) Activation of Na^+-H^+ exchange is necessary for RhoA-induced stress fiber formation. J. Biol. Chem. **271:** 22281–22284

70 Tominaga T. and Barber D. L. (1998) Na-H exchange acts downstream of RhoA to regulate integrin-induced cell adhesion and spreading. Mol. Biol. Cell. **9:** 2287–2303

71 Tominaga T., Ishizaki T., Narumiya S. and Barber D. L. (1998) p160ROCK mediates RhoA activation of Na-H exchange. EMBO J. **17:** 4712–4722

72 Lin X., Voyno-Yasenetskaya T. A., Hooley R., Lin C. Y., Orlowski J. and Barber D. L. (1996) Galpha12 differentially regulates Na^+-H^+ exchanger isoforms. J. Biol. Chem. **271:** 22604–22610

73 Baltz J. M., Biggers J. D. and Lechene C. (1991) Relief from alkaline load in two-cell stage mouse embryos by bicarbonate/chloride exchange. J. Biol. Chem. **266:** 17212–17217

74 Baltz J. M., Biggers J. D. and Lechene C. (1991) Two-cell stage mouse embryos appear to lack mechanisms for alleviating intracellular acid loads. J. Biol. Chem. **266:** 6052–6057

75 Baltz J. M. (1993) Intracellular pH regulation in the early embryo. Bioessays **15:** 523–530

76 Alper S. L. (1991) The band 3-related anion exchanger (AE) gene family. Annu. Rev. Physiol. **53:** 549–564

77 Yannoukakos D., Stuart-Tilley A., Fernandez H. A., Fey P., Duyk G. and Alper S. L. (1994) Molecular cloning, expression and chromosomal localization of two isoforms of the AE3 anion exchanger from human heart. Circ. Res. **75:** 603–614

78 Wang D. N. (1994) Band 3 protein: structure, flexibility and function. FEBS Lett. **346:** 26–31

79 Wang Z., Schultheis P. J. and Shull G. E. (1996) Three N-terminal variants of the AE2 Cl-/HCO_3^- exchanger are encoded by mRNAs transcribed from alternative promoters. J. Biol. Chem. **271:** 7835–7843

80 Stuart-Tilley A. K., Shmukler B. E., Brown D. and Alper S. L. (1998) Immunolocalization and tissue-specific splicing of AE2 anion exchanger in mouse kidney. J. Am. Soc. Nephrol. **9:** 946–959

81 Stuart-Tilley A., Sardet C., Pouyssegur J., Schwartz M. A., Brown D. and Alper S. L. (1994) Immunolocalization of anion exchanger AE2 and cation exchanger NHE-1 in distinct adjacent cells of gastric mucosa. Am. J. Physiol. **266:** C559–568

82 Kopito R. R., Lee B. S., Simmons D. M., Lindsey A. E., Morgans C. W. and Schneider K. (1989) Regulation of intracellular pH by a neuronal homolog of the erythrocyte anion exchanger. Cell **59:** 927–937

83 Linn S. C., Kudrycki K. E. and Shull G. E. (1992) The predicted translation product of a cardiac AE3 mRNA contains an N terminus distinct from that of the brain AE3 Cl^-/HCO_3^- exchanger. Cloning of a cardiac AE3 cDNA, organization of the AE3 gene, and identification of an alternative transcription initiation site. J. Biol. Chem. **267:** 7927–7935

84 Puceat M., Korichneva I., Cassoly R. and Vassort G. (1995) Identification of band 3-like proteins and Cl^-/HCO_3^- exchange in isolated cardiomyocytes. J. Biol. Chem. **270:** 1315–1322

85 Pucéat M. and Vassort G. (1996) Les échangeurs anioniques cardiaques: gènes multiples, protéines multiples, fonctions multiples. Méd. Sci. **12:** 1024–1028

86 Popov M., Tam L. Y., Li J. and Reithmeier R. A. (1997) Mapping the ends of transmembrane segments in a polytopic membrane protein. Scanning N-glycosylation mutagenesis of extracytosolic loops in the anion exchanger, band 3. J. Biol. Chem. **272:** 18325–18332

87 Tang X. B., Fujinaga J., Kopito R. and Casey J. R. (1998) Topology of the region surrounding Glu681 of human AE1 protein, the erythrocyte anion exchanger. J. Biol. Chem. **273:** 22545–22553

88 Lee B. S., Gunn R. B. and Kopito R. R. (1991) Functional differences among nonerythroid anion exchangers expressed in a transfected human cell line. J. Biol. Chem. **266:** 11448–11454

89 Brosius F. C. D., Alper S. L., Garcia A. M. and Lodish H. F. (1989) The major kidney band 3 gene transcript predicts an amino-terminal truncated band 3 polypeptide. J. Biol. Chem. **264:** 7784–7787

90 Kudrycki K. E. and Shull G. E. (1993) Rat kidney band 3 Cl^-/HCO_3^- exchanger mRNA is transcribed from an alternative promoter. Am. J. Physiol. **264:** F540–547

91 Richards S., Jaconi M., Vassort G. and Puceat M. J. Cell. Sci. (in press)

92 Puceat M., Roche S. and Vassort G. (1998) Src family tyrosine kinase regulates intracellular pH in cardiomyocytes. J. Cell Biol. **141:** 1637–1646

93 Jay D. G. (1996) Role of band 3 in homeostasis and cell shape. Cell **86:** 853–854

94 Harrison M. L., Rathinavelu P., Arese P., Geahlen R. L. and Low P. S. (1991) Role of band 3 tyrosine phosphorylation in the regulation of erythrocyte glycolysis. J. Biol. Chem. **266:** 4106–4111

95 Kay M. M. (1981) Isolation of the phagocytosis-inducing IgG-binding antigen on senescent somatic cells. Nature **289:** 491–494

96 Nilius B., Eggermont J., Voets T., Buyse G., Manolopoulos V. and Droogmans G. (1997) Properties of volume-regulated anion channels in mammalian cells. Prog. Biophys. Mol. Biol. **68:** 69–119

97 Motais R., Fievet B., Borgese F. and Garcia-Romeu F. (1997) Association of the band 3 protein with a volume-activated, anion and amino acid channel: a molecular approach. J. Exp. Biol. **200:** 361–367

98 Ding Y., Casey J. R. and Kopito R. R. (1994) The major kidney AE1 isoform does not bind ankyrin (Ank1) in vitro. An essential role for the 79 NH2-terminal amino acid residues of band 3. J. Biol. Chem. **269:** 32201–32208

99 Wang C. C., Moriyama R., Lombardo C. R. and Low P. S. (1995) Partial characterization of the cytoplasmic domain of human kidney band 3. J. Biol. Chem. **270:** 17892–17897

100 Chen J. H., Vijayakumar S., Li X. Q. and Alawqati Q. (1998) Kanadaptin is a protein that interacts with the kidney but not the erythroid form of band 3. J. Biol. Chem. **273:** 1038–1043

101 Gunn R. B., Dalmark M., Tosteson D. C. and Wieth J. O. (1973) Characteristics of chloride transport in human red blood cells. J. Gen. Physiol. **61:** 185–206

102 Zhang Y., Chernova M. N., Stuart-Tilley A. K., Jiang L. and Alper S. L. (1996) The cytoplasmic and transmembrane domains of AE2 both contribute to regulation of anion exchange by pH. J. Biol. Chem. **271:** 5741–5749

103 Muller-Berger S., Karbach D., Kang D., Aranibar N., Wood P. G., Ruterjans H. et al. (1995) Roles of histidine 752 and glutamate 699 in the pH dependence of mouse band 3 protein-mediated anion transport. Biochemistry **34:** 9325–9332

104 Muller-Berger S., Karbach D., Konig J., Lepke S., Wood P. G., Appelhans H. et al. (1995) Inhibition of mouse erythroid band 3-mediated chloride transport by site-directed mutagenesis of histidine residues and its reversal by second site mutation of Lys 558, the locus of covalent H2DIDS binding. Biochemistry **34:** 9315–9324

105 Sekler I., Lo R. S. and Kopito R. R. (1995) A conserved glutamate is responsible for ion selectivity and pH dependence of the mammalian anion exchangers AE1 and AE2. J. Biol. Chem. **270:** 28751–28758

106 Ganz M. B., Boyarsky G., Sterzel R. B. and Boron W. F. (1989) Arginine vasopressin enhances pHi regulation in the presence of HCO_3^- by stimulating three acid-base transport systems. Nature **337:** 648–651

107 Yanaka A., Carter K. J., Goddard P. J. and Silen W. (1992) Prostaglandin stimulates $Cl(-)$-HCO_3^- exchange in amphibian oxynticopeptic cells. Am. J. Physiol. **262:** G44–49

108 Harada H., Kanai Y., Anzai M. and Suketa Y. (1991) cAMP activates Cl-/HCO3-exchange for regulation of intracellular pH in renal epithelial cells. Biochim. Biophys. Acta **1092:** 404–407

109 Alvaro D., Della Guardia P., Bini A., Gigliozzi A., Furfaro S., La Rosa T., Piat C. and Capocaccia L. (1995) Effect of glucagon on intracellular pH regulation in isolated rat hepatocyte couplets. J. Clin. Invest. **96:** 665–675

110 Desilets M., Puceat M. and Vassort G. (1994) Chloride dependence of pH modulation by beta-adrenergic agonist in rat cardiomyocytes. Circ. Res. **75:** 862–869

111 Puceat M., Clement O. and Vassort G. (1991) Extracellular MgATP activates the Cl^-/HCO_3^- exchanger in single rat cardiac cells. J. Physiol. (Lond.) **444:** 241–256

112 Yu H. and Ferrier J. (1995) Osteoclast ATP receptor activation leads to a transient decrease in intracellular pH. J. Cell. Sci. **108:** 3051–3058

113 de Souza L. R., Moore H., Raha S. and Reed J. K. (1995) Purine and pyrimidine nucleotides activate distinct signalling pathways in PC12 cells. J. Neurosci. Res. **41:** 753–763

114 Yannoukakos D., Meyer H. E., Vasseur C., Driancourt C., Wajcman H. and Bursaux E. (1991) Three regions of erythrocyte band 3 protein are phosphorylated on tyrosines: characterization of the phosphorylation sites by solid phase sequencing combined with capillary electrophoresis. Biochim. Biophys. Acta **1066:** 70–76

115 Yannoukakos D., Vasseur C., Piau J. P., Wajcman H. and Bursaux E. (1991) Phosphorylation sites in human erythrocyte band 3 protein. Biochim. Biophys. Acta **1061:** 253–266

116 Harrison M. L., Isaacson C. C., Burg D. L., Geahlen R. L. and Low P. S. (1994) Phosphorylation of human erythrocyte band 3 by endogenous p72syk. J. Biol. Chem. **269:** 955–959

117 Watts J. D., Brabb T., Bures E. J., Wange R. L., Samelson L. E. and Aebersold R. (1996) Identification and characterization of a substrate specific for the T cell protein tyrosine kinase ZAP-70. FEBS Lett. **398:** 217–222

118 Ludt J., Tonnessen T. I., Sandvig K. and Olsnes S. (1991) Evidence for involvement of protein kinase C in regulation of intracellular pH by $Cl^-/HCO3^-$ antiport. J. Membr. Biol. **119:** 179–186

119 Camilion de Hurtado M. C., Alvarez B. V., Perez N. G., Ennis I. L. and Cingolani H. E. (1998) Angiotensin II activates Na^+-independent $Cl—HCO_3^-$ exchange in ventricular myocardium. Circ. Res. **82:** 473–481

120 Liu S., Piwnica-Worms D. and Lieberman M. (1990) Intracellular pH regulation in cultured embryonic chick heart cells. $Na(+)$-dependent Cl^-/HCO_3^- exchange. J. Gen. Physiol. **96:** 1247–1269

121 Alpern R. J. (1990) Cell mechanisms of proximal tubule acidification. Physiol. Rev. **70:** 79–114

122 Durand T., Gallis J. L., Masson S., Cozzone P. J. and Canioni P. (1993) pH regulation in perfused rat liver: respective role of $Na(+)$-H^+ exchanger and $Na(+)$-HCO_3^- cotransport. Am. J. Physiol. **265:** G43–50

123 Brune T., Fetzer S., Backus K. H. and Deitmer J. W. (1994) Evidence for electrogenic sodium-bicarbonate cotransport in cultured rat cerebellar astrocytes. Pflugers Arch. **429:** 64–71

124 Rajendran V. M. and Binder H. J. (1994) Differential modulation of Na-HCO_3 cotransport and Na-H exchange by pH in basolateral membrane vesicles of rat distal colon. J. Biol. Chem. **269:** 156–160

125 Boussouf A., Lambert R. C. and Gaillard S. (1997) Voltage-dependent $Na(+)$-HCO_3^- cotransporter and Na^+/H^+ exchanger are involved in intracellular pH regulation of cultured mature rat cerebellar oligodendrocytes. Glia **19:** 74–84

126 Romero M. F., Hediger M. A., Boulpaep E. L. and Boron W. F. (1997) Expression cloning and characterization of a renal electrogenic Na^+/HCO_3^- cotransporter. Nature **387:** 409–413

127 Newman E. A. (1991) Sodium-bicarbonate cotransport in retinal Muller (glial) cells of the salamander. J. Neurosci. **11:** 3972–3983

128 Newman E. A. and Astion M. L. (1991) Localization and stoichiometry of electrogenic sodium bicarbonate cotransport in retinal glial cells. Glia **4:** 424–428

129 Newman E. A. (1996) Acid efflux from retinal glial cells generated by sodium bicarbonate cotransport. J. Neurosci. **16:** 159–168

130 Deitmer J. W. and Schlue W. R. (1989) Functional role of the sodium-bicarbonate cotransport of glial cells in the leech central nervous system. Acta Physiol. Scand. Suppl. **582:** 31

131 Fitz J. G., Lidofsky S. D. and Scharschmidt B. F. (1993) Regulation of hepatic $Na(+)$-HCO_3^- cotransport and pH by membrane potential difference. Am. J. Physiol. **265:** G1–8

132 Ishiguro H., Steward M. C., Wilson R. W. and Case R. M. (1996) Bicarbonate secretion in interlobular ducts from guinea-pig pancreas. J. Physiol. (Lond.) **495:** 179–191

133 Romero M. F., Fong P. Y., Berger U. V., Hediger M. A. and Boron W. F. (1998) Cloning and functional expression of rNBC, an electrogenic Na^+-HCO_3^- cotransporter from rat kidney. Am. J. Physiol. **43:** F425–F432

134 Burnham C. E., Amlal H., Wang Z., Shull G. E. and Soleimani M. (1997) Cloning and functional expression of a human kidney Na^+:HCO_3^- cotransporter. J. Biol. Chem. **272:** 19111–19114

135 Amlal H., Wang Z., Burnham C. and Soleimani M. (1998) Functional characterization of a cloned human kidney Na^+:HCO_3^- cotransporter. J. Biol. Chem. **273:** 16810–16815

136 Burnham C. E., Flagella M., Wang Z., Amlal H., Shull G. E. and Soleimani M. (1998) Cloning, renal distribution and regulation of the rat Na^+-HCO_3^- cotransporter. Am. J. Physiol. **274:** F1119–1126

137 Romero M. F., Fong P., Berger U. V., Hediger M. A. and Boron W. F. (1998) Cloning and functional expression of rNBC, an electrogenic $Na(+)$-HCO_3^- cotransporter from rat kidney. Am. J. Physiol. **274:** F425–432

138 Abuladze N., Lee I., Newman D., Hwang J., Boorer K., Pushkin A. et al. (1998) Molecular cloning, chromosomal localization, tissue distribution and functional expression of the human pancreatic sodium bicarbonate cotransporter. J. Biol. Chem. **273:** 17689–17695

139 Lagadic-Gossmann D., Buckler K. J. and Vaughan-Jones R. D. (1992) Role of bicarbonate in pH recovery from intracellular acidosis in the guinea-pig ventricular myocyte. J. Physiol. (Lond.) **458:** 361–384

140 Camilion de Hurtado M. C., Perez N. G. and Cingolani H. E. (1995) An electrogenic sodium-bicarbonate cotransport in the regulation of myocardial intracellular pH. J. Mol. Cell Cardiol. **27:** 231–242

141 Camilion de Hurtado M. C., Alvarez B. V., Perez N. G. and Cingolani H. E. (1996) Role of an electrogenic $Na(+)$-HCO_3^- cotransport in determining myocardial pH_i after an increase in heart rate. Circ. Res. **79:** 698–704

142 Ruiz O. S. and Arruda J. A. (1992) Regulation of the renal Na-HCO_3 cotransporter by cAMP and Ca-dependent protein kinases. Am. J. Physiol. **262:** F560–565

143 Lagadic-Gossmann D., Vaughan-Jones R. D. and Buckler K. J. (1992) Adrenaline and extracellular ATP switch between two modes of acid extrusion in the guinea-pig ventricular myocyte. J. Physiol. (Lond.) **458:** 385–407

144 Terzic A., Puceat M., Clement-Chomienne O. and Vassort G. (1992) Phenylephrine and ATP enhance an amiloride insensitive bicarbonate-dependent alkalinizing mechanism in rat single cardiomyocytes. Naunyn Schmiedebergs Arch. Pharmacol. **346:** 597–600

145 Kohout T. A. and Rogers T. B. (1995) Angiotensin II activates the Na^+/HCO_3^- symport through a phosphoinositide-independent mechanism in cardiac cells. J. Biol. Chem. **270:** 20432–20438

146 Yannoukakos D., Vasseur C., Driancourt C., Blouquit Y., Delaunay J., Wajcman H. et al. (1991) Human erythrocyte band 3 polymorphism (band 3 Memphis): characterization of the structural modification (Lys 56–Glu) by protein chemistry methods. Blood **78:** 1117–1120

147 Jarolim P., Palek J., Amato D., Hassan K., Sapak P., Nierse G. T. et al. (1991) Deletion in erythrocyte band 3 gene in malaria-resistant Southeast Asian ovalocytosis. Proc. Natl. Acad. Sci. USA **88:** 11022–11026

148 Jarolim P., Rubin H. L., Zhai S., Sahr K. E., Liu S. C., Mueller T. J. et al. (1992) Band 3 Memphis: a widespread polymorphism with abnormal electrophoretic mobility of erythrocyte band 3 protein caused by substitution AAG–GAG (Lys–Glu) in codon 56. Blood **80:** 1592–1598

149 Jarolim P., Rubin H. L., Brabec V., Chrobak L., Zolotanev A. S., Alper S. L. et al. (1995) Mutations of conserved arginines in the membrane domain of erythroid band 3 lead to a decrease in membrane-associated band 3 and to the phenotype of hereditary spherocytosis. Blood **85:** 634–640

150 Jarolim P., Murray J. L., Rubin H. L., Taylor W. M., Prichal J. T., Ballas S. K. et al. (1996) Characterization of 13 novel band 3 gene defects in hereditary spherocytosis with band 3 deficiency. Blood **88:** 4366–4374

151 Inaba M., Yawata A., Koshino I., Sato K., Takeuchi M., Takakuwa Y. et al. (1996) Defective anion transport and marked spherocytosis with membrane instability caused by hereditary total deficiency of red cell band 3 in cattle due to a nonsense mutation. J. Clin. Invest. **97:** 1804–1817

152 Bruce L. J., Cope D. L., Jones G. K., Schofield A. E., Burley M., Pouey S. et al. (1997) Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (Band 3, AE1) gene. J. Clin. Invest. **100:** 1693–1707

153 Jarolim P., Shayakul C., Prabakanan D., Jiang L., Stuart-Tilley A., Rubin H. L. et al. (1998) Autosomal dominant distal renal tubular acidosis is associated in three families with heterozygosity for the R589H mutation in the AE1 (Band 3) Cl^-/HCO_3^- exchanger. J. Biol. Chem. **273:** 6380–6388

154 Michail S., Collins J. F., Xu H., Kaufman S., Vanderhoof J. and Ghishan F. K. (1998) Abnormal expression of brush-border membrane transporters in the duodenal mucosa of two patients with microvillus inclusion disease. J. Pediatr. Gastroenterol. Nutr. **27:** 536–542

155 Karmazyn M. (1996) The sodium-hydrogen exchange system in the heart: its role in ischemic and reperfusion injury and therapeutic implications. Can. J. Cardiol. **12:** 1074–1082

156 Levitsky J., Gurell D. and Frishman W. H. (1998) Sodium ion/hydrogen ion exchange inhibition: a new pharmacologic approach to myocardial ischemia and reperfusion injury. J. Clin. Pharmacol. **38:** 887–897

157 Cox G. A., Lutz C. M., Yang C. L., Biemesderfer D., Bronson R. T., Fu A. et al. (1997) Sodium/hydrogen exchanger gene defect in slow-wave epilepsy mutant mice. Cell **91:** 139–148

158 Grinstein S., Woodside M., Waddell T. K., Downey G. P., Orlowski J., Pouyssegur J. et al. (1993) Focal localization of the NHE-1 isoform of the Na^+/H^+ antiport: assessment of effects on intracellular pH. EMBO J. **12:** 5209–5218

159 Berridge M. J. (1997) Elementary and global aspects of calcium signalling. J. Exp. Biol. **200:** 315–319