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Topical Review

Regulation of K-Cl Cotransport: from Function to Genes

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Abstract. This review intends to summarize the vast literature on K-Cl cotransport (COT) regulation from a functional and genetic viewpoint. Special attention has been given to the signaling pathways involved in the transporter's regulation found in several tissues and cell types, and more specifically, in vascular smooth muscle cells (VSMCs). The number of publications on K-Cl COT has been steadily increasing since its discovery at the beginning of the 1980s, with red blood cells (RBCs) from different species (human, sheep, dog, rabbit, guinea pig, turkey, duck, frog, rat, mouse, fish, and lamprey) being the most studied model. Other tissues/cell types under study are brain, kidney, epithelia, muscle/smooth muscle, tumor cells, heart, liver, insect cells, endothelial cells, bone, platelets, thymocytes and Leishmania donovani. One of the salient properties of K-Cl-COT is its activation by cell swelling and its participation in the recovery of cell volume, a process known as regulatory volume decrease (RVD). Activation by thiol modification with N-ethylmaleimide (NEM) has spawned investigations on the redox dependence of K-Cl COT, and is used as a positive control for the operation of the system in many tissues and cells. The most accepted model of K-Cl COT regulation proposes protein kinases and phosphatases linked in a chain of phosphorylation/dephosphorylation events. More recent studies include regulatory pathways involving the phosphatidyl inositol/protein kinase C (PKC)-mediated pathway for regulation by lithium (Li) in low-K sheep red blood cells (LK SRBCs), and the nitric oxide (NO)/cGMP/protein kinase G (PKG) pathway as well as the platelet-derived growth factor (PDGF)-mediated mechanism in VSMCs. Studies on VSM transfected cells containing the PKG catalytic domain demonstrated the participation of this enzyme in K-Cl COT regulation. Commonly used vasodilators activate K-Cl COT in a dose-dependent manner through the NO/cGMP/PKG pathway. Interaction between the cotransporter and the cytoskeleton appears to depend on the cellular origin and experimental conditions. Pathophysiologically, K-Cl COT is altered in sickle cell anemia and neuropathies, and it has also been proposed to play a role in blood pressure control. Four closely related human genes code for KCCs (KCC1-4). Although considerable information is accumulating on tissue distribution, function and pathologies associated with the different isoforms, little is known about the genetic regulation of the KCC genes in terms of transcriptional and post-transcriptional regulation. A few reports indicate that the NO/cGMP/PKG signaling pathway regulates KCC1 and KCC3 mRNA expression in VSMCs at the post-transcriptional level. However, the detailed mechanisms of post-transcriptional regulation of KCC genes and of regulation of KCC2 and KCC4 mRNA expression are unknown. The K-Cl COT field is expected to expand further over the next decades, as new isoforms and/or regulatory pathways are discovered and its implication in health and disease is revealed.

Key words: K-Cl cotransport — Erythrocytes — Vascular smooth muscle cells — KCC genomic organization and expression — Signal transduction

Introduction

K-Cl cotransport (COT) is the electroneutral-coupled movement of K and C1 ions[1]. Since the system was first described as a swelling-[101, 199, 201] and thiol-

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[1]For the sake of simplicity, ions are typed without the corresponding valences.

activated [220] Cl-dependent K transport in red blood cells, numerous studies have confirmed its existence in several tissues during its "pre-genomic era", and have dealt with its kinetic, thermodynamic, metabolic, biochemical and functional properties both in health and disease. Earlier aspects of this work have been addressed in several reviews [15, 45–48, 54, 64, 68, 88, 98, 106, 108, 145, 146, 157, 173, 185, 204, 205, 214, 217, 258, 287, 320, 345, 355, 371]. The main conclusion from these reports is that K-Cl COT is ubiquitously present, involved in cell volume regulation, and, in turn, regulated by numerous activators and inhibitors. For example, cell swelling, thiol oxidation, cellular Mg-depletion, slight acidification (by lowering pH to 7), protein kinase inhibitors and free radicals are activators, whereas cell shrinkage, most bivalent cations, marked acidification (below pH 6.5), alkalinization (above pH 7.4), polyamines and protein phosphatase inhibitors are inhibitory. Furthermore, the system is highly activated in sickle cell anemia and plays an important role in neuropathies, as described below in section III. Following its cloning in 1996 [131, 293], several genes encoding the K-Cl cotransporter, and their multiple isoforms, have been found in most, if not all, tissues. Indeed, a new wave of information reveals the cotransporter's ubiquity and relevance in ion and volume homeostasis, as well as in the maintenance of life, as shown by animal models of gene ablation [26, 37, 47, 48, 64, 68, 88, 98, 103, 145, 146, 174, 214, 250, 345, 371].

Figure 1 shows a summary of a series of 331 published articles/reviews on K-Cl cotransport as a function of time, from 1980, year of its discovery, until 2002. This summary includes most of the studies/reviews dealing either exclusively or mainly with K-Cl cotransport and also those where the system is just mentioned for comparative purposes. Not included in this series are articles where K-Cl cotransport is called Cl-dependent K flux, which was its original name. It is likely that this growth will continue as more functional relevance under physiological and pathophysiological conditions is found.

Figure 2 shows the number of studies on K-Cl cotransport according to major cell types/tissues for the series represented in Figure 1. Obviously, most of the studies were done primarily in **red blood cells**, including those in **human** [24, 29, 30, 47, 49, 52, 63, 75, 77, 100, 101, 112, 123–125, 130, 135, 139, 142, 158, 175–177, 187, 205, 216, 219, 248, 257, 260, 269–271, 273, 275, 276, 281, 308, 311–313, 315, 334, 360], **sheep** [4–6, 23, 27, 28, 84–87, 96–99, 114, 116, 186, 196, 203, 205–209, 211–213, 215, 217, 218, 220, 221, 237, 283, 310, 369], **dog** [65–67, 118, 119, 198, 284–290], **rabbit** [50, 167, 168, 337], **horse** [53, 68, 126, 127, 254, 335, 336], **guinea pig** [362], **turkey** [254], **duck** [239], **frog** [9–11, 140, 141, 143], **rat** [95, 147, 159, 278], **mouse** [18, 19, 73, 74, 78, 297, 307, 330], **fish** [34, 35, 137, 172, 201, 345], and **lamprey** [194].

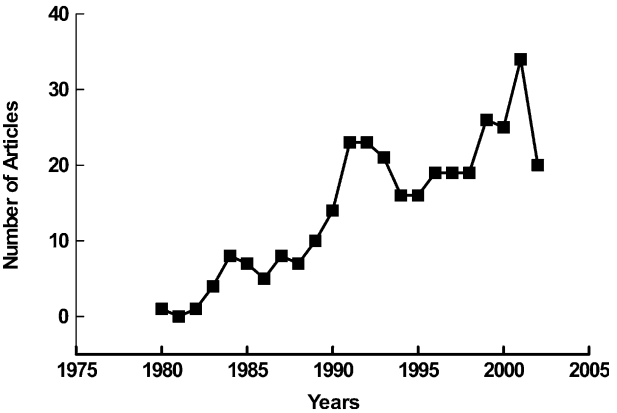

Fig. 1. Publications on K-Cl COT as a function of time. Number of publications from a sample of 331 published articles and reviews for the period 1980 to 2002. This sample includes articles and reviews dealing either exclusively or mainly with K-Cl COT and also those where the system is mentioned for comparative purposes. Not included, are studies where K-Cl COT is referred to as Cl-dependent K flux, as it was originally called.

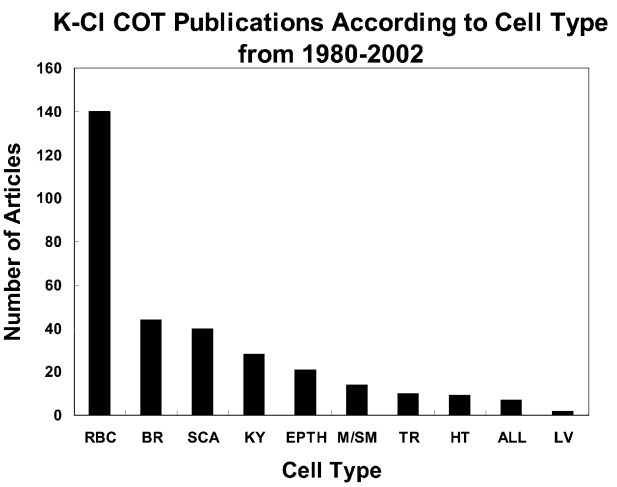

Fig. 2. Number of studies on K-Cl COT according to major cell types/tissues. Studies from the sample in Figure 1, covering the period 1980–2002, were classified in cell types and tissues. RBC, BR, SCA, KY, EPTH, M, HT, TR, LV represent red blood cells, brain, sickle cell anemia, kidney, epithelia, muscle, heart, tumor, and liver, respectively.

In the second place are studies on **brain tissue** [21, 42, 81, 82, 88, 111, 122, 138, 155, 156, 161, 162, 164, 165, 179, 180, 182, 184, 188, 229, 238, 255, 274, 277, 291–294, 303, 304, 316, 333, 339, 344, 347, 348, 354, 361, 363–365, 367, 375], followed by studies in erythrocyte transport in **sickle cell anemia** [37, 38, 45, 48, 50, 51, 54–59, 72, 74, 104, 109, 110, 128, 129, 154, 157, 173, 185, 210, 223, 226, 227, 244, 253, 256, 258, 268, 272, 282, 300, 305, 324, 341, 351, 352], and with decreasing frequency those in **kidney** [14, 15, 33, 39,

44, 61, 62, 102–107, 121, 132, 134, 136, 144, 153, 222, 230, 240, 242, 301, 325, 331, 349, 355, 357, 358], **epithelia** [20, 70, 71, 79, 94, 120, 146, 189, 190, 228, 234, 252, 317–319, 329, 342, 346, 353, 370], **muscle/smooth muscle** [2, 7, 8, 17, 64, 89, 91–93, 115, 280, 314, 356, 373], **tumor cells** [12, 83, 160, 171, 191–193, 197, 225, 247, 295], **heart** [13, 16, 150, 157, 163, 195, 299, 343, 368], studies where K-Cl cotransport is reported to be present in **most (all) cells/tissues** [131, 145, 214, 249, 251, 320, 371], and in **liver** [25, 245]. Not shown are studies in **insect cells** [40, 259], **endothelial cells** [296], **bone** [148], **platelets** [80], **thymocytes** [113], and **Leishmania donovani** [31].

Figures 1 and 2 indicate that, due to its role in cellular homeostasis, that is, constancy of cellular volume and chemical composition of intracellular compartments, the K-Cl cotransporter may be involved in multiple processes essential for cell survival. Given the large scope of activities in the field, this review has been divided in three major areas. The first one deals with the regulation of K-Cl cotransport in enucleated and nucleated erythrocytes and in other cell types and tissues. It also includes work done in disease states and on the relationship between the transporter and the cytoskeleton. The second area describes some of the information available on the regulation of K-Cl cotransport in vascular smooth muscle cells. This includes general properties, regulation by signaling cascades, as summarized in a working model, and the newly uncovered potential role of the transporter in vasodilation. The final and third major area summarizes information available on the regulation of the transporter at the gene level and concentrates specifically in the human KCC genes and in the genomic organization and expression of the presently known isoforms: KCC1, KCC2, KCC3 and KCC4. This section not only summarizes the available information but also provides predictions based on *in silico* analysis for some of the different sub-isoforms.

Outline of Review:

Cellular Regulation of K-Cl Cotransport

- Erythrocytes from Several Species
- Nonerythrocyttc Cell Types
- Regulation of K-Cl Cotransport in Disease States
- Relationship between K-Cl Cotransport and the Cytoskeleton

K-Cl Cotransport in Vascular Smooth Muscle Cells

- General Properties
- Regulation by Signaling cascades
- Model of K-Cl Cotransport Regulation by Signal Transduction
- K-Cl Cotransport and Vasodilation

Regulation at the Gene Level and Molecular Biology of Human KCC Genes

- General Aspects
- Genomic Organization and Expression
  - *KCC1*
  - *KCC2*
  - *KCC3*
  - *KCC4*

Cellular Regulation of K-Cl Cotransport

Lipid-protein interactions are essential for cellular function and occur at different levels of cellular organization [3]. The advent of molecular biology in the last decades has accelerated both the quest for and knowledge of the mechanisms involved in cellular and inter-cellular communication. At the center of interest are the mechanisms of signal transduction [3]. These are multi-step processes involving hormones or growth factors, cellular membrane components (proteins, lipids) working as transducers, and a cascade of enzymes, cofactors and molecules interacting amongst each other and with cellular structures (cytoskeleton, organelles, macromolecules, polymers) (*see* [149] for further information). Proteins have been known as the main components of signaling pathways. However, one of the emerging areas of intensive research is concerned with the role of lipids as signals, intermediaries or products of a signaling cascade. An example is the newly demonstrated existence of lipid rafts or lipid microenvironments on the cell surface that incorporate proteins involved in signal transduction cascades. A salient characteristic of lipid rafts is that the inclusion or exclusion of proteins is dependent on the signaling pathway and that in response to intra- or extra-cellular stimuli, they can change their composition and size [332].

Erythrocytes from Several Species

Ion transporters are recognized components of intracellular signaling pathways [149] and those that are activated by cell swelling have been proposed to operate as receptors, messengers and effectors [320]. For example, the K-Cl cotransporter is activated by cell swelling and in turn regulates cell volume through a process known as regulatory volume decrease (RVD) (Fig. 3 and *see* Table 1) [106, 204, 217]. This phenomenon has been demonstrated in numerous studies done in normal red blood cells (RBCs). Some of the findings on K-Cl COT regulation in RBCs are summarized below and have been separated according to species, since inter-species differences have been observed. Similarities or differences in K-Cl COT response to manipulations or regulation of the

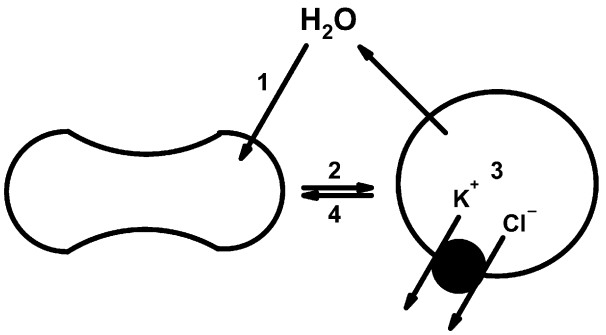

Fig. 3. Representative scheme of regulatory volume decrease (RVD). In step 1, suspension of cells in hypotonic medium causes water to move in and, consequently, cell swelling (step 2). This process activates volume-sensitive transporters, in this example K-Cl COT, which extrude K and Cl with obligatory outward movement of water (step 3). Upon completion of this process, the cells recover their original volume (step 4). This last step may occur at different rates depending on the cell type or tissue.

system by effectors will be indicated whenever the information is available (*see* also Table 1).

Human RBCs possess a swelling- [30, 101, 124, 187, 269, 276, 311], NEM-activated [29, 124, 216, 219], and DIOA-sensitive K-Cl COT efflux [124]. The activation by cell swelling is strongly inhibited by cytochalasin B, suggesting participation of the cytoskeleton in the regulatory response of K-Cl COT [124].

Several regulators of K-Cl COT activity have been proposed for human RBCs. In RBC ghosts, soluble polycations such as spermine and methyl glyoxal, and cationic amphiphiles (such as sphingosine and tetracaine) inhibit K-Cl COT independently of cell volume. These compounds are thought to regulate K-Cl COT in ghosts through negative charges (phosphatidylserine or phosphatidylinositides) located at the inner membrane surface, thus excluding the possibility of cellular metabolic regulation [311]. However, evidence for metabolic regulation in sheep and human RBCs was previously reported [202, 219] and regulation of K-Cl COT through a metabolic cascade was proposed by Jennings and Al-Rohil for rabbit red cells [169] and by cAMP in RBCs from other species (*see* references in [217]). Further evidence for regulation of K-Cl COT through a cascade of kinases and phosphatases has been reported in cells from different tissues. In particular, serine/threonine and tyrosine kinases/phosphatases have been proposed in the regulatory pathway/s. For example, in human RBCs, protein phosphatase inhibitors inhibit K-Cl COT [178, 279]. Furthermore, K-Cl COT activity and membrane-bound protein phosphatase type 1 and 2A (PP1 and PP2A, respectively) activities are positively correlated, suggesting participation of these enzymes in K-Cl COT modulation by cell swelling, NEM, and Mg_i-depletion [29, 30]. There is also evidence supporting regulation of K-Cl COT by a volume-sensitive kinase [187], first proposed by Jennings and his group to inhibit K-Cl COT [169, 170] (*see* Fig. 4). Thus, the evidence available indicates that signal transduction pathways regulate K-Cl COT and that swelling activation may also occur without involvement of cellular metabolism.

In dog RBCs, Mg_i and Li_i regulate the response of K-Cl COT to swelling [290]. As in human and rabbit RBCs, activation and deactivation of volume-sensitive K-Cl COT appears to be regulated by phosphorylation/dephosphorylation events [288]. The response of K-Cl COT to volume changes, together with that of Na/H exchange, led to the proposal that osmotic changes modulate enzyme activity, a phenomenon more generally known as "molecular crowding" [287, 289]. The high-potassium (HK) dog RBC is an interesting model for studying the role of K-Cl COT in cell volume regulation [118]. In addition to a high K content, these cells also possess high glutathione (GSH), and when separated by their density, in contrast to human and sheep RBCs [214, 217], the oldest cells are lightest and the younger cells are heavier. In addition, the K-Cl COT activity is higher in younger and lower in older cells, as it is in human and sheep RBCs [214, 217]. Protection of K-Cl COT activity and cell volume regulation from thiol modification, requires an intact redox system, rather than high GSH alone [119].

Low-K (LK) sheep RBCs (SRBCs) constitute one of the first models to study K-Cl COT, since mature cells possess an active system, in contrast to most other cell types where the system is latent and requires activation [204, 214, 217]. A significant amount of information about its properties and regulation has been obtained in sheep RBCs [214, 217]. These cells and those of other artiodactyla, such as goat, and cattle, as well as those of some members of the order carnivora, such as cats and dogs (*see* above) are characterized by a cation dimorphism, i.e., the HK or LK steady state. Studies on membrane thiol groups of LK and HK sheep RBCs revealed a similar distribution of these groups in the two types of cells and gave support to the hypothesis that their difference in K-Cl COT activity is due to cytoplasmic control of the transporter [202, 209, 214, 217], as it was described above for human, rabbit and dog RBCs. The suggestion of K-Cl COT control by a cytoplasmic cascade in sheep RBCs has been further supported by the finding that staurosporine, a protein kinase inhibitor, activates the transporter in these cells [27, 116]. Activation of K-Cl COT by staurosporine requires that a putative kinase inhibits the phosphatase that regulates the transporter [27, 116]. Oxidants have been shown to stimulate K-Cl COT [204, 214, 217].

Table 1. Summary of activators and inhibitors, regulation pathways, regulators, function and pathology of K-Cl COT

Cell/Tissue	Activators	Inhibitors	Regulators/Pathways	Function	Pathology	References[1]
Enucleated Erythrocytes:						
Human	Swelling NEM Mg_i-depletion Diamide Acidification Urea	Cytoch. B[2] DIOA Polycations Cat. Amphiph. Okadaic acid Calyculin A	PKs/PPs VK PP1 PP-2A Hb C HB OARAB Src, Syk	RVD	SCA Cell Dehyd.	5,29,30,37,38, 54,57,59,72,77, 100,123,177, 185,216 217,226,243, 256,268,279 311
Dog	swelling	Mg_i Li_i Molecular crowding	PKs/PPs	RVD		118,119,214 217,284–290
Sheep	Swelling Staurosporine Low pH Oxidants: (SH-R, H_2O_2, NO_2^-, NO) Vasodilators: (HYZ, SNP,ISSB,PE,$NaNO_2$)	Calyculin A Genistein KT5823 Li_i, DIDS PD98059 $H^+{}_i$ SH-R	PKs/PPs PI Pathway PKC MEK		SCA model	1,4,6,7,27,28, 113,115,201, 203–209,211– 215,217
Rabbit	Swelling NEM		PKs/PPs PP1 VK			167–170 214,217,337
Rat	Swelling High Temp.	Okadaic acid Calyculin A	PKs/PPs Cytoskeleton VK	RVD		278
Mouse	Diamide Swelling		Src Ks: Fgr, Hck Prot. 4.2 Hb C, Hb S, Hb γ	RVD	SCA model	5,18,73,88, 297,306,340
Guinea Pig	High Temp.					362
Equine	Oxygenation CO, NO_2^-	Deoxygenation				53,127
Nucleated Erythrocytes:						
Duck, frog, Fish	Swelling High O_2 HO^-	Thiols Low Ta. NaF Vanadate	PKs/PPs	RVD		11,34,68,137,199 201,239
Non-erythrocytes:						
HEK-293, Brain, Heart, Epithelia, Tumor Cells	Swelling NEM	Cl_i Furos. (> 1 mM) Bumet. (> 100 μM)	SPAK OSR1	RVD Cl_i regulation K absorption/ homeostasis Apoptosis	Neuropath., M.I.	81,83,88,106 131,145,155, 156,163,189, 190,192,195, 197,217,222, 274,277,291, 295,299,318, 343,355,368

[1]References correspond to cell type or tissue but not to individual activators, inhibitors, regulators, function and/or associated pathology.
[2]Abbreviations used: (in order of appearance in the Table): Cytoch B, Cytochalasin B; Pks/PPs, protein kinases/protein phosphatases; RVD, regulatory volume decrease; SCA, sickle cell anemia; NEM, N-ethylmaleimide; DIOA, [(dihydroindenyl) oxy] alkanoic acid; VK, volume-sensitive kinase; Cell Dehyd., cell dehydration; PP1, protein phosphatase 1; Cat. Amphiph., cationic amphiphiles; PP-2A, protein phosphatase 2A; Hb, hemoglobin; Mg_i, internal Mg; Li_i, internal Li; PI Pathway, phosphatidyl inositol pathway; PKC, protein kinase C; MEK, mitogen-activated protein kinase kinase; H_2O_2, hydrogen peroxide; NO_2^-, nitrite; NO, nitric oxide; HYZ, hydralazine; SH-R, thiol reagents; SNP, sodium nitroprusside; ISSB, isosorbide mononitrate; PE, pentaerythritol; HO; Hydroxyl radical; Temp., temperature; Src Ks, Src kinases; Prot. 4.2, protein 4.2; CO, carbon monoxide; Furos., furosemide; Bumet., bumetanide; SPAK, Ste-20-related proline-alanine-rich kinase; OSR1, oxidative stress response 1 kinase; Neuropath., neuropathies; M.I., myocardial ischemica.

Amongst these compounds, H_2O_2 appears to stimulate the phosphatase/s involved in K-Cl COT activation [28]. At least two phosphorylation sites on the transporter or regulator have been proposed, based on the additive effect of NEM and H_2O_2 [28] and MgATP depletion studies [283]. In fact, evidence of regulation by the PP1 and PP2A phosphatases exists for human red cells [29, 30]. However, it is also possible that the two compounds act through different signaling pathways.

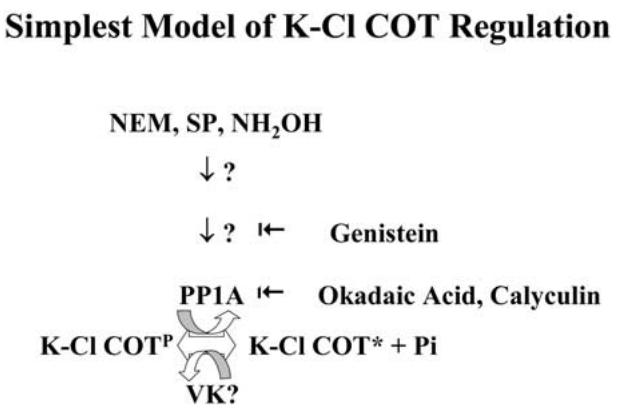

Fig. 4. Simplest model of K-Cl COT regulation. This scheme summarizes the most commonly proposed mechanism for K-Cl COT regulation by thiol reagents such as N-ethyl maleimide (NEM) and hydroxylamine (NH_2OH) and for drugs that are serine/threonine enzyme inhibitors such as staurosporine (SP). Genistein is a tyrosine kinase inhibitor, calyculin and okadaic acid are serine/threonine phosphatase inhibitors of protein phosphatase 1 and 2A (PP1 and PP2A), respectively.

For instance, the effect of the oxidant nitrite (NO_2^-) on K-Cl COT was first found in LK SRBCs [6]. NO_2^- is a stable metabolic end product of the short-lived highly reactive free radical nitric oxide (NO), an oxidant, modulator of ion channels, and potent vasodilator. In some systems, the response to NO_2^- is identical to that of NO. We hypothesized that NO_2^- activates K-Cl COT. The effect of various concentrations (10^{-6} to 10^{-1} M) of $NaNO_2$ was studied on K efflux in hypotonic Cl and NO_3 media, Cl-dependent K efflux, glutathione (GSH), and met hemoglobin (MetHb) formation. Increasing concentrations of $NaNO_2$ stimulated K efflux and K-Cl COT. Stimulation of K efflux was dependent upon external Cl, a characteristic of K-Cl COT. A lag phase consistent with kinase/phosphatase activation of K-Cl COT was also detected. Exposure of LK SRBCs to $NaNO_2$ decreased GSH, an effect characteristic of a thiol-oxidizing agent and confirmed by concomitant MetHb formation. Activation of K-Cl COT was positively correlated with MetHb formation. N-ethylmaleimide (NEM) was used to assess the mechanism of NO_2^- action. These early findings suggested that NEM and NO_2^- utilize at least one common pathway for K-Cl COT activation, and that K-Cl COT might be involved in cardiovascular disease [6]. In separate studies, commonly used vasodilators such as hydralazine (HYZ), sodium nitroprusside (SNP), isosorbide mononitrate (ISSB) and pentaerythritol activated K-Cl COT in a concentration-dependent manner in LK SRBCs incubated in media of different osmolalities (240–450 mOsM) [1, 6, 7, 372] and in the presence and absence of calyculin or genistein, inhibitors of the cotransporter, and of KT5823, a selective inhibitor of PKG [7]. All vasodilators activated K-Cl COT in these cells, and calyculin and genistein inhibited this activation, whereas KT5823 abolished the sodium nitroprusside-stimulated K-Cl-COT, suggesting involvement of the cGMP pathway in K-Cl COT activation [7]. Furthermore, the data indicate that cell swelling alters activation of K-Cl COT by the vasodilators tested and that the mechanism of activation is drug-dependent and may involve the cellular metabolism [372].

On the other hand, lithium and protein kinase C (PKC) modulators regulate swelling-activated K-Cl COT in sheep red blood cells (SRBCs), with involvement of the phosphatidylinositol pathway (*see* above, and [114]. Lithium increased the production of diacylglycerol in a bimodal fashion, without altering the phosphatidylinositol concentration, and revealing the presence of a complete PI cycle in LK SRBCs. In addition, phorbol ester treatment and inhibition of mitogen-activated protein kinase (ERK1/2) kinase by PD98059 and of serine/threonine phosphatases by calyculin inhibit, in a time-dependent fashion, swelling-activated K-Cl COT [114] (Fig. 5). These results suggest that modulation of the cellular phospholipid metabolism affects the response of K-Cl COT to changes in cellular volume [114] (*see* Fig. 6). As in dog RBCs [290], internal but not external Li inhibits K-Cl COT activity in sheep RBCs. This inhibitory effect could result from Li acting on an allosteric site on the transporter and /or its regulators [114]. In addition, Li appears to alter the response of K-Cl COT to pH and volume changes by modulation of the cellular phospholipid metabolism and a PKC-dependent regulatory pathway [114].

Rabbit RBCs are also a good model to study K-Cl COT regulation. Studies in these cells support earlier evidence of metabolic regulation [202, 214, 217], and proposed participation of a regulatory kinase/phosphatase cascade [169, 170]. Furthermore, these studies provided the first evidence that PP1 is involved in K-Cl COT regulation [337].

In mouse RBCs, K-Cl COT is involved in RVD [18] and is activated by diamide [5]. Another important finding in terms of K-Cl COT regulation is the fact that RBCs of knockout mice for the Src family kinases, Fgr and Hck, had significantly higher transport activity in the double mutant erythrocytes with respect to the wild type [76]. This family of kinases plays a central role in the regulation of hematopoietic cell functions. Thus, Fgr and Hck kinases appear to inhibit the phosphatase/s that activate/s the transporter [76]. Regulation of K-Cl COT is altered in protein 4.2–null mice [297]. This protein is a major component of the RBC membrane skeleton and its deletion significantly increases transport activity [297]. The mouse K-Cl cotransporter KCC1 has been recently cloned and expression in oocytes [340] indicates that the system displays the

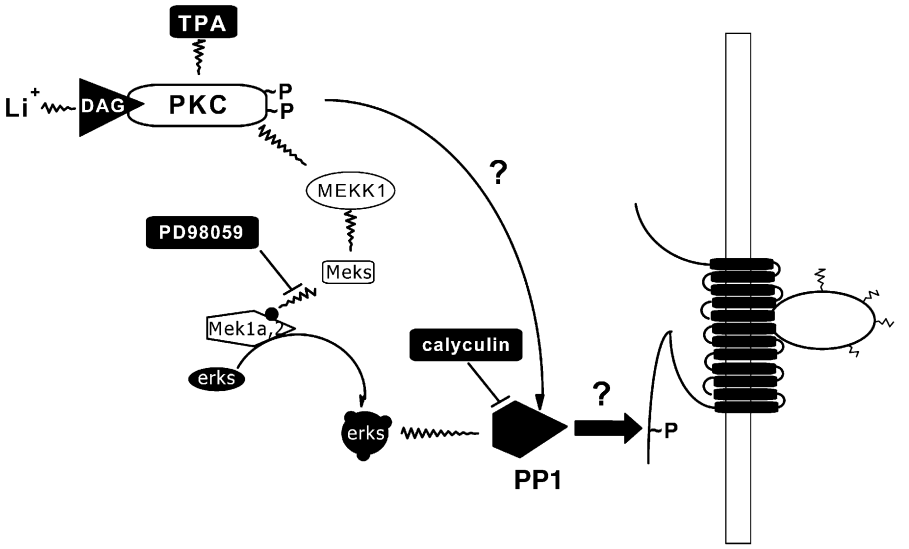

Fig. 5. Proposed mechanism of K-Cl COT regulation by internal lithium (Li_i). Li increases diacylglycerol (DAG), substrate of protein kinase C (PKC), which is involved in the transmission of the signal to one of several cascades of protein serine/threonine kinases that utilize the sequential phosphorylation of kinases to transmit and amplify the signal. These kinase cascades are collectively known as mitogen-activated protein kinase (MAPK) signaling cascades. One of the best studied of these kinase cascades is the ERK (p44/p42 MAPK) signaling cascade. The intermediates are MAPK kinase kinase (MEKK1), MAPK kinases (Meks), Mek1a/2, and erks. Downstream targets of erk include p90rsk (p90 ribosomal S6 protein kinase), and the Elk-1 and Stat3 transcription factors (not shown in the scheme). This cascade is proposed to activate serine/threonine phosphatases (PP1 and/or PP2-A). Although the phorbol ester TPA acts as a substrate of PKC, stimulation of K-Cl COT is blocked by an unknown mechanism (not indicated in this scheme). PD98059 and calyculin are inhibitors of the MAPK pathway and of PP1, respectively.

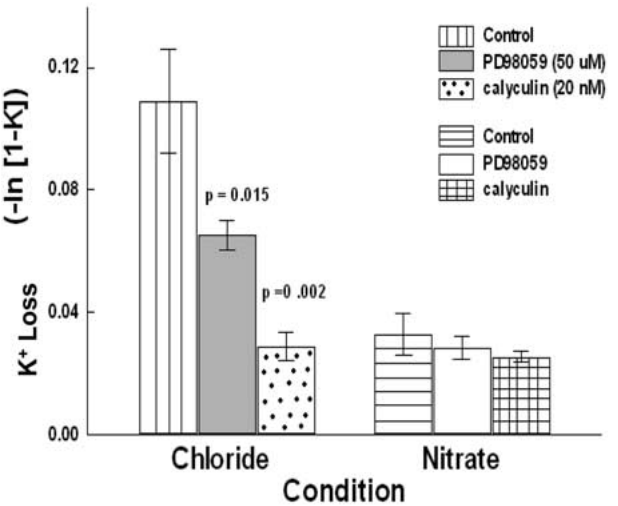

Fig. 6. Effect of PD98059 and calyculin on K loss in low K sheep red blood cells (LK SRBCs). RBCs were incubated in the presence and absence of the MEK kinase inhibitor, PD98059 (50 μM) or calyculin (20 nM) in Cl and NO_3 medium [114] and the fractional K loss calculated as 1n (1–*K*). Statistical significance was determined with respect to the control.

known characteristics of K-Cl COT described in other cells [214, 217].

Mild warming of guinea pig RBCs (from 37°C to 41°C) activates K-Cl COT [362]. Although some of the properties of the transporter are similar to those described in RBCs from other species [214, 217], the kinetics of activation of the system by mild warming led to conclude that regulation of guinea pig RBC K-Cl COT is altered to compensate for ionic imbalances caused by the increase in temperature [362]

Equine RBCs possess a K-Cl COT with characteristics similar to those found in sheep and normal human RBCs [53]. As in other cells [214, 217], oxygenation of equine RBCs activates, and deoxygenation deactivates K-Cl COT [53]. Other oxidants such as carbon monoxide and nitrite also activate K-Cl COT in these cells. The degree of activation by these compounds with respect to oxygenated RBCs is commensurate with MetHb formation and GSH depletion [128], as described previously for sheep RBCs [6].

Some nucleated cells such as duck, frog and fish RBCs possess a swelling activated K-Cl COT [68, 199, 201]. In duck RBCs, the response of K-Cl COT to cell swelling [199, 239], is similar to that observed in human, dog, sheep and several other species. Furthermore, cell volume and internal Cl (Cl_i) modulate the activity of the transporter through involvement of kinases and phosphatases and phosphorylation/dephosphorylation events [239], similarly to what has been reported for other species. In many enucleated and nucleated RBCs, K-Cl COT is susceptible to inhibition by physical parameters such as

osmotic pressure and temperature and by ions or chemical compounds [214, 217]. NaF inhibits K-Cl COT in frog and sheep RBCs [11, 214] and vanadate, in frog and rabbit RBCs [11, 169], and this inhibition may also involve protein phosphatases [11]. K-Cl COT in trout RBCs is activated by incubation of cells under high O_2 tension, an effect also reported for equine RBCs ([68] and references therein, [34]). A putative transduction pathway also involves a metabolic cascade to account for activation by oxygenation [68]. However, an alternative mechanism by which O_2 may modulate K-Cl COT activity in trout is by release of hydroxyl radical from O_2 in the vicinity of the cell membrane [34].

Non-erythrocytic Cell Types

In the late 1980s, it was clear that many cells, including renal cells, had the capability to downregulate their volume after swelling [106]. However, the signal transduction pathways were largely unknown [106]. In polarized cells such as renal cells, the K-Cl cotransporter is expressed in the basolateral membrane and plays an important role in transcellular ion movement [355]. Cultured human embryonic kidney (HEK)-293 cells are used as a model to study kidney cells in vitro. Some properties of K-Cl COT in HEK-293 cells over-expressing the K-Cl cotransporter 1 (KCC1) are similar to those observed in red cells [132]. However, some differences have been reported in terms of their response to staurosporine, hydroxylamine, NEM, cell swelling and pH changes [222]. Furthermore, the signal transduction pathways involved in K-Cl COT regulation in these cells have not been reported yet.

Studies in cultured astrocytes and rat midbrain neurons, and in pyramidal neurons from rat neocortical slices have revealed the presence of K-Cl COT [81, 156, 164, 274, 291]. In astrocytes, K-Cl COT participates in RVD [274, 291], whereas in cultured rat midbrain neurons, the transporter plays a dual role in the control of Cl_i. Under normal external K (K_o) concentrations, the cotransporter is outwardly directed. At high K_o, as observed during neuronal hyperactivity, K-Cl COT is inwardly directed and accumulates Cl. This increase in Cl_i further increases neuronal excitability [164]. Similar results were found in mature rat neocortical pyramidal neurons but not in neurons from neonatal rats, further stressing the important role of K-Cl COT in the homeostasis of cellular Cl and its regulation during development [81]. The K-Cl cotransporter also interacts with the stress-related kinases Ste-20-related proline-alanine-rich kinase (SPAK) and oxidative stress response 1 (OSR1) both in vivo and in vitro, suggesting that this type of interaction may be involved in cellular stress signaling [298]. It is expected that in the near future more detailed studies on the signal transduction pathways that regulate K-Cl COT in brain cells will be conducted.

Piwnica-Worms et al. provided the first evidence for the presence of K-Cl COT in heart by measuring ^{36}Cl fluxes in a polystrand preparation of cultured chick heart cells [299]. Since then, several reports have confirmed the existence of K-Cl COT in various heart preparations [157, 195, 343, 368]. In all preparations, the system is recognized as a volume regulator. Furthermore, during myocardial ischemia, the transporter is activated in response to myocyte volume regulation, suggesting an important role for the system under this condition [368].

Besides RBCs, epithelial cells were one of the first systems where presence of K-Cl COT was reported (*see* references in [217]). In epithelia, the properties of K-Cl COT are similar to those described for other cell types and the major function of the system appears to be regulation of cell volume [146, 190, 318]. In rat colonic epithelium, K-Cl COT has been proposed to mediate transepithelial K absorption and to be involved in K homeostasis by "sensing" the levels of dietary K [318].

The role of K-Cl COT in volume regulation has also been investigated in tumor cells [83, 191, 193, 197, 277, 295]. The cells studied are derived from Ehrlich ascites tumor [197], human and mouse with erythroleukemia [83, 295], HepG2 human hepatoblastoma [191, 193] and N1E mouse neuroblastoma [277]. Involvement of K-Cl COT in RVD appears to be independent of the cellular origin and conditions of study, whereas its involvement in apoptosis is not [191, 193, 277].

Further studies on the presence, function and regulation of K-Cl COT in different tissues has been summarized elsewhere [68, 96, 145, 214, 217, 320, 371].

Regulation of K-Cl Cotransport in Disease States

Due to its ubiquitous nature and prominent role in cellular homeostasis, evidence of abnormal structure, function and/or regulation of the transporter is expected to be found under pathological conditions as research progresses in the field. One of the first and most studied diseases where this transporter has been found operationally abnormal is sickle cell anemia. In RBCs homozygous for hemoglobin S and C and heterozygous SC, K-Cl COT is elevated with respect to normal hemoglobin AA cells. The higher activity was observed under several experimental conditions (swelling, NEM treatment and acidification) [59]. The increased activity of the transporter contributes to increased mean corpuscular hemoglobin and subsequent cell dehydration [54, 57]. Separation of SS cells in density-defined fractions shows that its activity disappears after reticulocyte maturation [57]. However, mathematical modeling and further

experimentation uncovered a novel mechanism of cell dehydration, where K-Cl COT plays an important role. This created the fast-track hypothesis of irreversible sickle cells directly formed from reticulocytes [37, 38, 227]. Studies in transgenic mouse red cells indicate that Hb C and Hb S+gamma affect both quantitatively and qualitatively K-Cl COT by altering its activity and/or regulation [306]. In RBCs from knockout mice for the Src family kinases, the Fgr and Hck kinases appear to inhibit the phosphatase/s that activate/s the K-Cl cotransporter [76]. Recent studies in deoxygenated sickle RBCs indicate that Syk (a Syk family protein kinase) and Src regulate K-Cl COT through opposing yet interconnected effects [243]. Furthermore, an increasing number of reports indicate that abnormalities in K-Cl COT can cause from mild to severe neuropathies in human and murine species [88]. A more detailed description of these abnormalities will be given below under Genomic Regulation.

Relationship between K-Cl Cotransport and the Cytoskeleton

The K-Cl Cotransporter regulates cell volume through stimulation by cell swelling, a process known as RVD. In spite of the abundant literature on K-Cl COT, only few studies have dealt with the interaction between the transporter and the cellular cytoskeleton. Some of these studies suggest either direct or indirect interactions, whereas a few others find independent responses upon modulation of these cellular structures. For instance, interaction between the cotransporter and the cytoskeleton occurs when cell volume sensors, by detecting deformation or stretching of the cellular membrane and its cytoskeleton upon swelling, activate K-Cl COT and induce RVD [320]. Human RBCs containing HbC and HB0ARAB have an abnormally elevated K-Cl COT activity, which may be brought about by interaction of these hemoglobins with the red cell membrane, its cytoskeleton or both [257]. Furthermore, interaction between the cotransporter and the cytoskeleton is supported by inhibition of swelling-activated DIOA-sensitive K-Cl COT by cytochalasin B in human RBCs [124], and by the finding that 10-min thermal pre-treatment of rat erythrocytes at 49 °C causes full-scale activation of K-Cl cotransport and blocks its regulation by swelling, a temperature at which spectrin denaturation also occurs [278]. Furthermore, the cytoskeleton network plays a key role in volume-dependent K-Cl cotransport activation in human and rat erythrocytes, suggesting involvement of protein phosphorylation-dephosphorylation events, as determined with the phosphoprotein inhibitors okadaic acid and calyculin [279]. Independence between modulation of the cotransporter and the cytoskeleton appears to exist in hereditary spherocytosis [77] and in human red cells with a cytoskeletal deficiency [5].

Table 1 summarizes some activators, inhibitors and regulators of K-Cl cotransport as well as the functions and associated pathologies in the different cell types/tissues described in this section. From the data in Table 1, it is apparent that some cells/tissues (human and sheep red cells) have received more attention than others (non-erythrocytes). However, lately, there has been an exponential growth in the area of brain research and K-Cl cotransport regulation as well as on the role K-Cl cotransport abnormalities play in the manifestation of disease (*see* below).

KCl Cotransport in Vascular Smooth Muscle Cells

General Properties

In many cells, K-Cl COT is activated by cell swelling, thiol reagents, such as NEM and diamide, and by internal Mg depletion, whereas it is inhibited by cell shrinkage, okadaic acid, calyculin and genistein ([214, 217] and references therein). The system has been extensively characterized in RBCs from a kinetic and thermodynamic standpoint. In contrast, little is known about its properties in VSMCs. In the A10 cell line, K-Cl COT is activated by cell swelling, it is involved in volume regulation and is inhibited by DIOA [314]. However, DIOA has been reported to exert diverse side-effects in VSMCs, including cytotoxicity [17], and thus may not be used as a criterion for K-Cl cotransport identification, at least in nucleated cells. In addition, inhibitors such as furosemide, okadaic acid and calyculin A are unable to inhibit the swelling-activated K-Cl COT in these cells [17].

Amongst the thiol reagents, NEM has been extensively used as an activator of K-Cl COT [197, 217, 220]. Thus, NEM was used in rat aortic VSMCs to test for presence of a functional K-Cl COT, and as a positive control for the response to the vasodilator HYZ [7]. Incubation of primary cultures of VSMCs with 0.5 mM NEM or 1.75 mM HYZ activated a Cl-dependent Rb influx, indicating that these cells possess K-Cl COT. The activation by NEM was 13.2-fold and that by HYZ 10.4-fold with respect to the control [7]. Characterization of K-Cl COT in VSMCs is in progress as well as identification of the different regulatory pathways (*see* below).

Regulation by Signaling Cascades

Several transport pathways have been proposed as transducers of hormone actions in VSMCs and vascular endothelial cells [43, 261–267]. Furthermore, as described above, ion transporters and cyclic nucleo-

tides are recognized components of intracellular signaling pathways ([149] and references therein). In VSMCs, nitrovasodilators relax contracted VSM both in vitro and in vivo. These drugs release NO, which at submicromolar concentrations activates the soluble guanylyl cyclase (GCs) that catalyzes the conversion of GTP into cGMP. This nucleotide activates protein kinase G (PKG), which is involved in the modulation of ion transport pathways [149, 231, 232] and references therein, [359] (*see* Figure 7). However, at micromolar concentrations of NO or higher, other NO-signaling mechanisms independent of elevations in cGMP have been described [133, 166, 181, 231, 232, 321]. Furthermore, cGMP can regulate cellular function by activation of PKG or by acting directly on non-selective ion channels such as in the vertebrate rod photoreceptor cell as well as renal and olfactory epithelial channels [231, 232] and the Ca-dependent K channels in VSMCs [36].

Of particular interest in the cGMP-dependent NO-signaling pathway is the role of PKG in the regulation of ion transport. This kinase, which comprises a group of closely related enzymes, is a serine/threonine protein kinase and belongs to the protein kinase superfamily. Numerous and varied substrates have been reported for this protein [231, 232], which possesses two catalytic site inhibitors, H-8 and KT5823, with reported K_i values for the purified enzyme of 0.48 μM and 0.23 μM, respectively. These inhibitors also inhibit cAMP-dependent protein kinase but with K_i one order of magnitude higher [231, 232]. Both, repetitive passage of cultured rat aortic SMCs and continuous exposure of cultured bovine aortic SMCs to NO donors, are associated with loss of expression of PKG [233]. Presence or absence of PKG determines the phenotype of VSMCs. Thus, PKG-containing cells possess a contractile-like morphology, whereas PKG-deficient cells become dedifferentiated and "synthetic" [32].

In the last decades, a wealth of information has been accumulated on the complex and precise mechanisms that control ion channels and, to a lesser extent, of those that control ion transporters (exchangers and cotransporters). Of particular interest here is the regulation of K-Cl COT, which is proving to be more complex than expected, since several signaling cascades appear to be involved. The NO-dependent, cGMP-mediated signaling pathway in VSMCs has been extensively investigated [231, 232]. However, the transducers at the membrane level remain largely unknown. Cultured VSMCs obtained from rat aorta possess a K-Cl COT activity that is stimulated by NEM and HYZ [7]. Activation of VSMC K-Cl COT by HYZ is inhibited by calyculin and genistein, resembling the behavior of LK SRBCs [7]. Furthermore, activation of VSM K-Cl COT by the vasodilators HYZ and SNP decreases, in a dose-dependent manner, VSM tension in isolated porcine

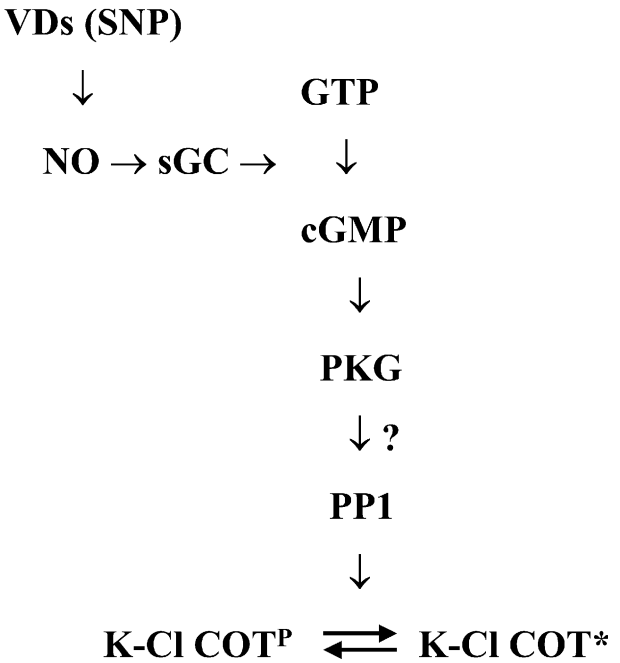

Fig. 7. Proposed scheme for the regulation of K-Cl COT by vasodilators in vascular smooth muscle cells (VSMCs). Vasodilators (VDs) such as sodium nitroprusside (SNP) release nitric oxide (NO) and activate the soluble guanylyl cyclase (sGC). This enzyme converts GTP into cGMP and the nucleotide activates protein kinase G (PKG), which by an unknown mechanism activates protein phosphatase 1 (PP1) and activates K-Cl COT.

coronary rings. Relaxation of pre-contracted arteries occurred endothelium-independently when only K-Cl COT was operating and other pathways for K/Rb transport, including the Ca-activated K channel, were inhibited [7]. Newer vasodilators known as NONOates, which are NO releasing drugs, also activate K-Cl COT in VSMCs. These findings suggested regulation of K-Cl COT by the NO/cGMP/PKG pathway in VSMCs (*see* Fig. 7) *and* a role for K-Cl COT in vasodilation.

The role of PKG on K-Cl COT regulation was further investigated in PKG-transfected (PKG+) and PKG-deficient (PKG-) SMCs (Fig. 8), in the presence and absence of 0.5 mM NEM, and as a function of the NEM concentration (0–0.5 mM). Results showed that cells containing PKG have higher baseline K-Cl COT, a different profile for NEM activation and display a different inhibition profile for the NEM-activated component by furosemide and DIOA when compared to PKG cells, indicating a role for PKG on KCl COT regulation in VSMCs [7].

The activity of PKG decreases after repetitive passages of rat aortic SMCs and concomitantly, the cells loose a contractile-like phenotype to become "synthetic", [32, 233]. We have found that the NEM- and HYZ-induced activation of K-Cl COT in VSMCs also decreased after several passages of rat aortic SMCs, whereas the basal K-Cl COT was independent

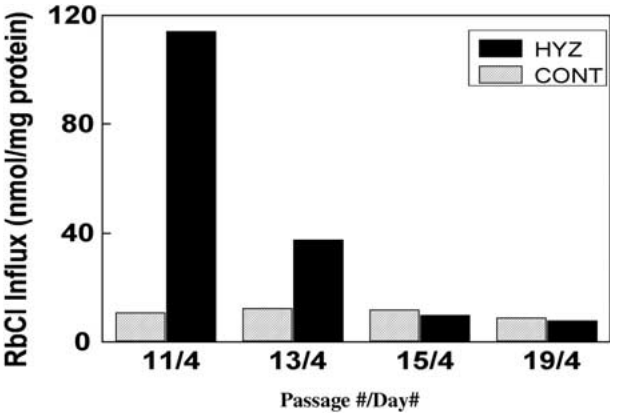

Fig. 8. Effect of VSMC age (passage number) on the activation of RbCl influx by hydralazine (HYZ). VSMCs at passages 11 to 19 were cultured for 4 days. Cl-dependent (sulfamate substitution) Rb influx was measured as described elsewhere [7], in the presence and absence of HYZ. *Striped bars*: control (*CONT*), *black bars*: 1.75 mM HYZ (*HYZ*).

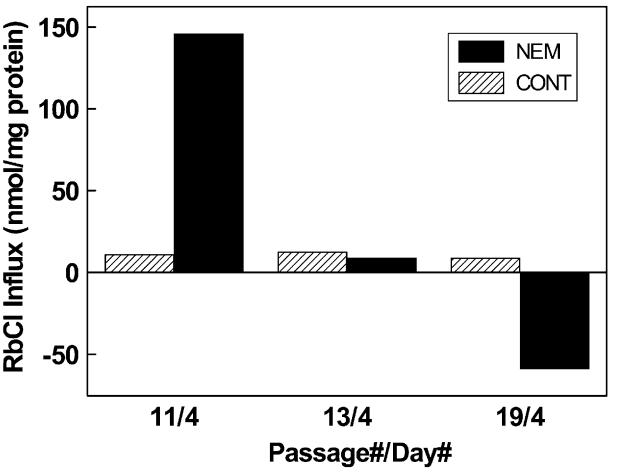

Fig. 9. Effect of VSMC age (passage number) on the activation of RbCl influx by NEM. VSMCs at passages 11 to 19 were cultured for 4 days. Cl-dependent (sulfamate substitution) Rb influx was measured as described elsewhere [7], in the presence and absence of NEM. *Striped bars*: control (*CONT*), *black bars*: 0.5 mM NEM.

of the passage number. These results are shown in Figs. 8 and 9. Figure 8 shows the RbCl influx as a function of passage number and after 4 days in culture (day #) both for control (*striped bars*) and for HYZ (1.75 mM)-treated cells (*black bars*). Similar results were found for NEM-activated RbCl influx in Fig. 9. In this figure the negative values of RbCl influx indicate that the flux in Cl-free medium (sulfamate replacement) was larger than in Cl. The conclusion of these experiments is that the HYZ- and NEM-stimulated RBCl influx (K-Cl COT) appears to be different from the basal activity of the system. Because HYZ and NEM are not NO donors and, based on the studies on K-Cl regulation by the NO/cGMP/PKG pathway [6–8], the results of Figs. 8 and 9 seem to indicate that cellular aging may also affect a common regulator of the system, likely protein kinases or phosphatases or both. In light of the findings with PKG, this point clearly needs further investigation.

Besides the regulation of K-Cl COT by Li through the phosphatidyl inositol and the NO/cGMP/PKG pathways in LK SRBCs and VSMCs [6–8, 89, 90, 92, 93, 114, 373], a more recently uncovered regulatory mechanism for K-Cl COT involves serum factors. Serum and its growth factors control the activity of several ion channels and transporters. In VSMCs, serum-starvation (24 h) prior to flux measurement abolished basal K-Cl COT. No effect of serum-starvation was observed in cells treated with 0.05 mM NEM. In contrast, hypotonically swollen VSMCs exhibited K-Cl COT in serum-starved but not in serum-fed cells [372], suggesting that serum or serum factors are necessary for functional expression of K-Cl COT in VSMCs. Platelet-derived growth factor (PDGF), a potent serum mitogen for VSMCs, plays an important role in membrane transport regulation and in arteriosclerosis. In primary cultures of VSMCs, addition of PDGF increases total protein content and K-Cl COT activity in a time-and dose-dependent manner. PDGF activates K-Cl COT both acutely (10 min) and chronically (12 h). AG1296, a selective inhibitor of the PDGF receptor tyrosine kinase, abolishes these effects [373]. The PDGF-dependent signaling pathways involved in K-Cl COT regulation in VSMCs is presently under investigation [373].

Model of K-Cl Cotransport Regulation by Signal Transduction

Our latest interpretation of K-Cl COT regulation by signal transduction pathways in RBCs and VSMCs is depicted in the model of Fig. 10. This shows the "classical", regulation pathway for NEM, staurosporine (SP) and hydroxylamine (NH_2OH) and the newly discovered pathways for vasodilators (VDs (NO), Li and PDGF [1, 3, 6–8, 89, 91–93, 372, 374]. It remains to be demonstrated whether these pathways also regulate the transporter at the protein level. This subject is presently under investigation. Likewise, inhibition of K-Cl COT by Li was reported in LK SRBCs and involves the phosphatidyl inositol pathway and likely PKC [114]. However, the role of Li in VSMCs remains to be determined. In addition, the signal transduction pathway involving PDGF [373, 374] is presently under investigation and appears to involve the PI3K signaling cascade [374].

K-Cl Cotransport and Vasodilation

The findings of an effect of commonly used vasodilators on K-Cl COT in LK SRBCs and VSMCs through the NO/cGMP/PKG pathway [6, 7, 89, 92,

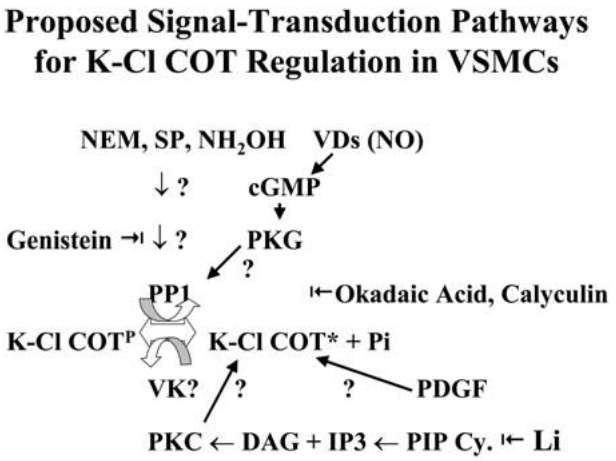

Fig. 10. Proposed signal transduction pathways for K-Cl COT regulation in VSMCs. This scheme summarizes, in addition to the most commonly proposed mechanisms for K-Cl COT regulation, the most recently reported signal transduction pathways uncovered in LK SRBCs and in VSMCs. The NO/cGMP/PKG pathway was described in the legend to Fig. 7. The Li-regulated pathway involves inhibition of the phosphatidyl inositol phosphate cycle (*PIP Cy.*), increase in diacylglycerol (*DAG*) and inositol tri-phosphate (*IP3*), activation of PKC and inhibiton of K-Cl COT by an unknown mechanism. The platelet-derived growth factor (PDGF)-regulated pathway activates K-Cl COT as reported elsewhere [373, 374]. *VK*: volume-sensitive kinase.

93] led to propose a putative role of K-Cl COT in cardiovascular disease. To further test this hypothesis, we recently investigated the effect of KCC3 protein deletion by gene disruption [155] on blood pressure and heart rate [2]. Basal blood pressure was measured in KCC3 −/− and control +/+ mice with the Data Sciences telemetry system. The results showed that mice lacking the KCC3 protein exhibit a marked hypertension [2]. Some of the KCC3 −/− mice had systolic blood pressures in excess of 185 mmHg. There were no differences in heart rate between the groups. Measurement of water intake showed that consumption was similar between the groups (4.9 ± 0.4 *vs.* 6 ± 0.6 ml/24 h, control *vs.* KCC3−/−) [2]. These results provide direct physiological evidence for a role of K-Cl COT in vasodilation.

Regulation at the Gene Level and Molecular Biology of Human KCC Genes

All data concerning the molecular structure, genomic organization for *in silico* analysis to predict general molecular properties of all human KCC products were obtained from publicly available databases and web-based software located at: genomic.sanger.ac.uk, www.cbs.d u.dk, scansite.mit.edu, www.ensembl.org, www.ncbi.nlm.nih.gov, genome.ucsc.edu, www.fruitfly.org, argon.cshl.org, and pkr.sdsc.edu).

General Aspects

The human potassium chloride cotransporter (hKCC) genes belong to the inorganic cation chloride cotransporter (CCC) gene super family [145, 309]. Under certain experimental conditions, the KCC gene products can be pharmacologically distinguished from the rest of the CCC members as ouabain- and bumetanide-insensitive, furosemide-sensitive, Cl-dependent K transport. However, so far, there are no known specific inhibitors for the transporter. Four different human genes codifying for hKCCs have been identified: hKCC1–4, all of them mediating electroneutral transport [151, 153, 251, 293, 309, 340] as well as $NH4^+$ [22, 235]. All members of the hKCC gene family share a similar membrane topology: two hydrophilic N- and C-terminal intracellular domains connected by a central core of twelve hydrophobic transmembrane domains. A large extracellular loop connects the fifth and the sixth transmembrane domain and conserves three identical sites for N-linked glycosylation in all hKCC isoforms [251].

According to DNA sequence alignment and analysis, hKCCs are 70 %, 65 %, and 66 % identical to rKCC2, hKCC1, and hKCCC4, respectively. The hKCC4 is 74 %, and 66 % identical to hKCC1, and hKCC3, respectively. Hence four main hKCC variants form two groups, hKCC1/hKCC4 and hKCC2/hKCC3. Several sub-isoforms of the hKCC1 and hKCC3 isoforms were described: three full-length hKCC1 cDNAs encoding for C-terminally different KCC1s (hKCC*1a*, hKCC*1b*, and hKCC*1c*), and three hKCC3 cDNAs encoding for N-terminal variants of KCC3. *In silico* analysis identifying several potential phosphorylation sites, both conserved and non-conserved in all hKCC proteins, were detected by using PhosphoBase server (Fig. 11). Despite the fact that protein phosphorylation and dephosphorylation are proposed to play important roles in the regulation of red cell K-Cl cotransport [214], direct *in vivo* phosphorylation of KCC proteins has not been demonstrated yet.

The four main hKCC proteins are 65–71 % homologous with highly conserved transmembrane domains (Fig. 11). The cytoplasmic domains in the four types of hKCC proteins are significantly different in the distribution/presence of putative phosphorylation consensus sequences. Notably, the hKCC2 C-terminal tyrosine (Y^{1087}) is conserved in mKCC3 and hKCC3 (Y^{1054}) [251]. However, only hKCC1 protein sequences reveal predicted consensus phosphorylation sites at S^{51}, and Y^{108}, as well as one glycosylation site at D^{245}, and one myristoylation, at G^{239}.

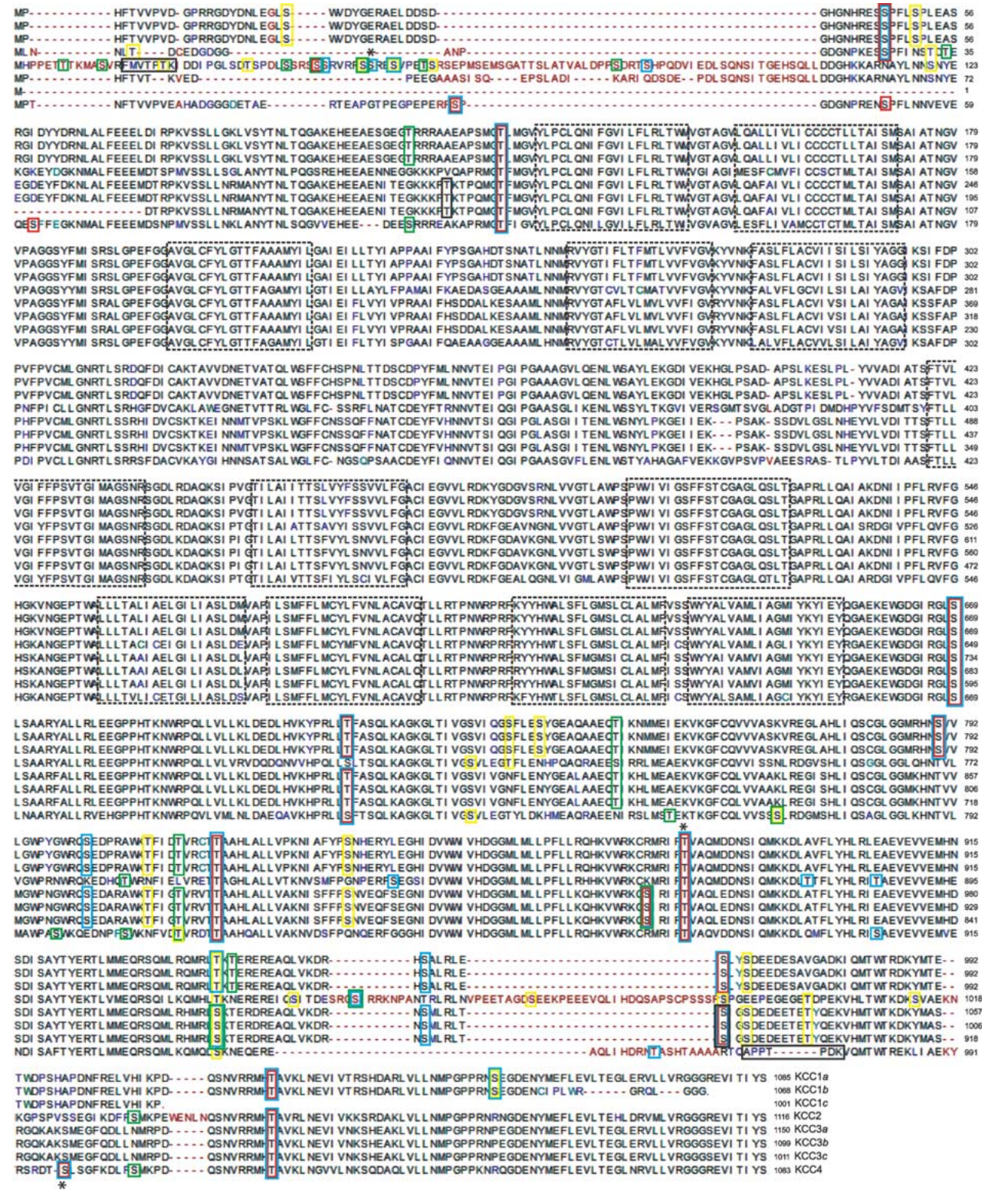

Fig. 11. Protein sequence alignment of hKCCs and predicted phosphorylation sites. The protein sequences of human KCC proteins (KCC1a, KCC1b, KCC1c, KCC2, KCC3a, KCC3b, KCC3c, and KCC4) translated from the corresponding cDNAs were aligned and shown using the single-letter code. Putative transmembrane domain sequences are included in dashed black squares. PhosphoBase database was used to search for consensus phosphorylation sites. Red letters represent insertions. Protein kinase A, C, and G consensus phosphorylation sites are indicated in blue, green, and black vertical squares, respectively. Calmodulin-dependent protein kinase II and casein kinase II consensus phosphorylation sites are shown in red and yellow vertical squares, respectively. p70S6K and p34cdc2 consensus phosphorylation sites are indicated by an asterisk and a horizontal black square, respectively.

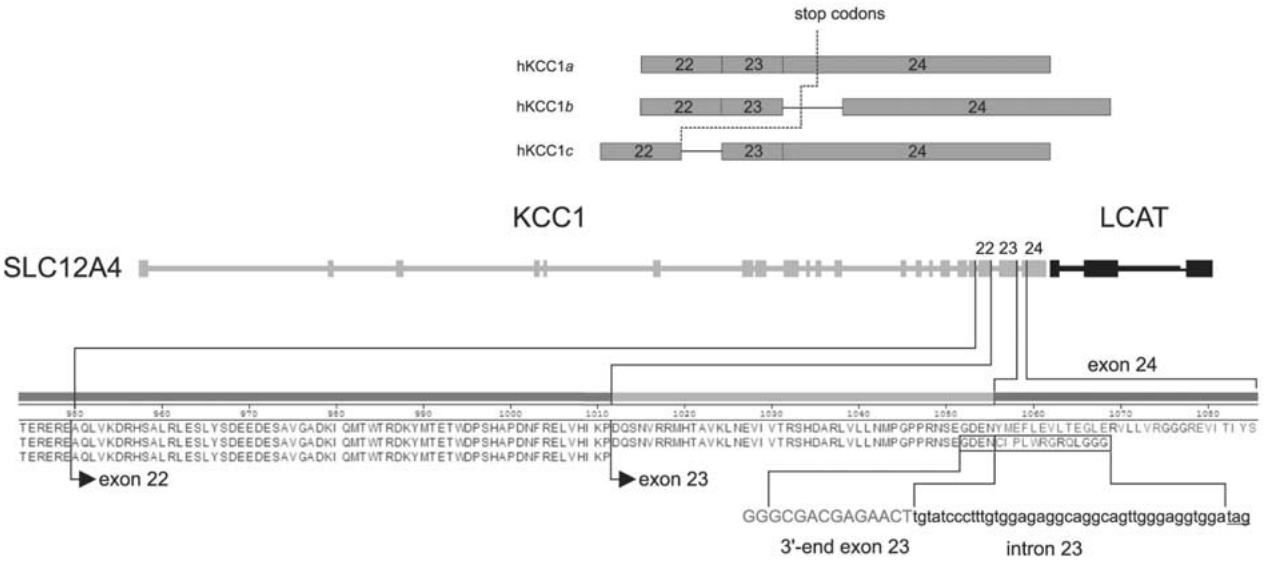

Fig. 12. Genomic organization of the hKCC1 gene. Diagram showing the genomic organization of the human KCC1 gene SLC12A4 located immediately at the 5′ LCAT gene, partially represented in black. *Upper panel:* Main differences between the alternatively spliced KCC1a, KCC1b, and KCC1c isoforms. The coding sequences are shown in gray boxes, and the respective relative position of stop codons are also indicated. *Lower panel:* Detailed sequences involved in the alternative splicing found in KCC1 isoforms.

The conserved cytoplasmic segments of all KCC isoforms were suggested to be involved in protein-protein interactions [251, 298], however, no recognizable protein signaling domains are present [323]

Genomic Organization and Expression

KCC1

Mammalian KCC1 is a strongly conserved gene since more than 96 % homology exists between human, rabbit, rat, sheep and mouse KCC1 proteins. The hKCC1 gene (locus SLC12A4), covering 28.8 kb, is located in the long arm (q22.1) of the chromosome 16 on the direct strand, and its 3′-end overlaps with the promoter region of the lecithin-cholesterol acyltransferase gene [117, 131]. The hKCC1 gene products are considered to be a housekeeping transport mechanism implicated in cell volume regulation, since it is widely expressed in mammalian tissues [131, 153, 214, 340]. Detailed *in silico* analysis of the hKCC1 gene reveals that 24 exons code for several hKCC1 mRNAs. At least 6 open reading frames encoding for almost identical hKCC1 proteins were found in human cDNA clones [131, 200, 295]. Moreover, the finding of 3′-end differences in hKCC1 cDNAs suggests that the hKCC1 gene possesses the potential to generate several 3′-unique mRNA isoforms (hKCC1a, hKCC1b, and hKCC1c). Different 3′-end open reading frames of hKCC1 cDNAs encode for 3 proteins differing at their C-terminals: 1086 amino acids in hKCC1a, 1068 amino acids in hKCC1b, and 1012 amino acids in hKCC1c (Fig. 12). The presence of different C-terminal regions in KCC1 proteins raises the possibility of differential posttranscriptional regulation. However, and with the exception of two absent residues in hKCC1c (T^{1021}, and S^{1050}), all hKCC1 isoforms (a, b and c) share the potential phosphorylation sites for CaM-II, CK-II, p70S6k, PKA, and PKC, as shown by *in silico* analysis using prediction algorithms based on substrate consensus sequences for the most popular protein kinases [366] (Fig. 11). No consensus sites were found for MLCK, p34cdc2, and PKG. Thus, direct phosphorylation of KCC1 *in vivo* remains to be demonstrated.

The relationship between the N- and C-terminal domains of mKCC1 and its K-Cl cotransport activity was recently determined. Both, the C-terminal and the membrane-proximate region of the N-terminal domain are necessary for transport function. A dominant negative inhibitor of wild-type KCC1 transport function was recently engineered by deletion of the entire N-terminal domain [60]. The dominant negative phenotype was caused by direct association of the mutant KCC1 with the wild-type KCC1, an interaction that requires the presence of the C-terminal domain [60]. Moreover, a truncation of the last C-terminal amino acids was enough to block mKCCl function, as well as the dominant negative phenotype. Truncation of the last N-terminally located 46 amino acids only diminished KCC1 function [60]. In addition, the removal from rabbit KCC1 of most of the C-terminal domain abolished activation by NEM in HEK-293-transfected cells

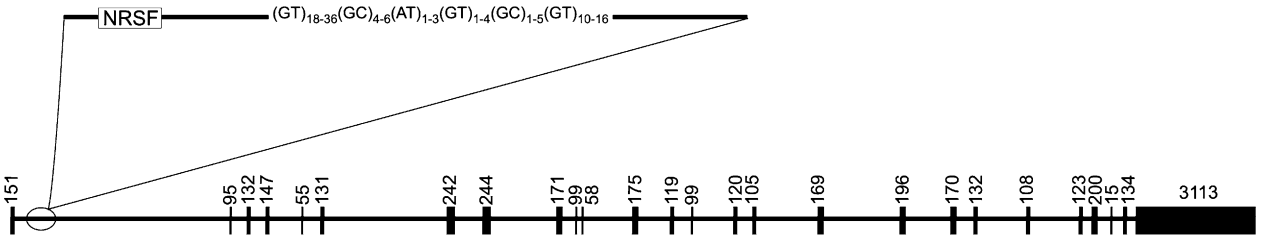

Fig. 13. Genomic organization of the hKCC2 gene. Shown is a representative diagram of the genomic organization of the human KCC2 gene (SLC12A5). Dark gray boxes represent each one of 26 exons of the gene, and its respective length in bp is also shown. Intronic sequences are represented as gray lines connecting each exon. Note detailed intronic region showing the relative positions of the putative sequence recognized by NRSF and the highly variable dinucleotide polymorphisms.

while apparently decreasing but not abolishing surface expression [221]. Shen et al., by using KCC1 dominant-negative mutants, demonstrated that K-Cl cotransport is an important modulator of growth and invasiveness of human cervical cancer [327].

Analysis and comparison of the 3′-end region of hKCC1 cDNAs reveal interesting features. The C-terminal region of the largest hKCC1a [131, 200] is encoded by the last 3 exons, whereas the two shortest hKCC1c and hKCC1c cDNAs appear to be the result of alternative intronic splicing of one of the 2 introns between the last 3 exons [295]. Indeed, exons 22, 23, and 24 plus an intronic sequence of 155 bp (intron 23) are included in the 3′-end sequence of hKCC1b cDNA. Furthermore, the first 39 bp from intron 23 code for the last 13 amino acids located in hKCC1b. On the other hand, the last 3 exons present in hKCC1c cDNA are interrupted by a short intronic sequence (intron 22), making exons 23 and 24 the 3′-untranslated region of hKCC1c (Fig. 12). The physiological significance, as well as tissue distribution and relative contribution of the hKCC1 isoforms to the total KCC1 pool remain to be determined.

The role of the promoter region in the transcriptional regulation of hKCC1 gene expression, the transcriptional initiation site/s, and/or the intronic characteristics has not been determined yet. However, the mouse KCC1 promoter sequence analysis reveals several features consistent with a housekeeping gene. For instance, several ubiquitous transcription factor-binding sites are located 5′ the first exon, but the classic TATA box and CCAAT motif are absent. Interestingly, a long perfect direct repeat with multiple CACCC motifs and a high GC content near the first codon probably indicates a role in transcriptional or translational regulation [340]. In addition, several single sequences repeat intronic polymorphisms and Alu-like sequences were also detected in mouse KCC1 intronic sequences [330].

Northern blot analysis of human tissues using a restriction fragment containing the 3′-untranslated region of KCC1 as a probe revealed that two KCC1 transcripts (3.8 kb and 4.4–5.6 kb) are ubiquitously expressed [131, 200]. However, the 3′-end versions of hKCC1 mRNAs were only detected in circulating reticulocytes [295]. Although hKCC1 is expressed in every cell tested so far, little is known about the genetic regulation of KCC1 mRNAs. RNA-polymerase II-independent, protein kinase G (PKG)-dependent upregulation of rat KCC1 gene expression was recently described in primary cultures of rat VSMCs [89]. The precise mechanisms involved in the PKG-dependent post-transcriptional regulation of KCC1 mRNA are unknown. Furthermore, the relevance of NO per se in the upregulation of KCC1 mRNA was recently demonstrated [92]. Interestingly, only rapid NO releasers were able to increase KCC1 mRNA to a similar extent, suggesting that the rate of NO release plays an important role in KCC1 mRNA upregulation [92].

KCC2

Human KCC2 is encoded by 26 exons located in the SLC12A5 gene on 32 kb of the direct DNA strand in chromosome 20, cytogenetic marker q13.12 [316, 333]. According to the sequence published in GeneBank® (NT_011362.7), the hKCC2 gene possesses the potential to generate at least 8 different mRNAs predicted to encode for 8 different proteins. However, only two full-length human cDNAs encoding for identical hKCC2 proteins were cloned so far (AF208159 and NM_020708). The intron-exon boundaries and the size of each intron present in the hKCC2 gene were corroborated recently [333]. The first intron in the hKCC2 gene extends for almost 5 kb and contains a complex and variable dinucleotide repeat polymorphism and a potential binding site for a silencing factor restrictive to neurons (NRSF) [184, 333] (Fig. 13). This 21-bp sequence shares 80% homology to the consensus site for neuronal-restrictive silencing factor binding [322] and confers transcriptional silencing in non-neuronal cells [184].

The translated hKCC2 cDNA sequence produces a protein of 1116 amino acids, with a predicted molecular weight of 123.5 kDa. Protein sequence analysis revealed that hKCC2 possesses several po-

tential phosphorylation sites for CaMK-II, PKA, and PKC (*see* Fig. 11). *In silico* analysis predicts that the unique C-5 terminal insertion in hKCC2 is encoded by exons 22 and 23, and was suggested to play a role in maintaining the functional conformation of KCC cotransporters and/or to be involved in essential regulatory protein-protein interactions [339].

Northern blot and RT-PCR analysis showed that KCC2 transcript expression appears to be restricted to neurons throughout the central nervous system, retina, CA1-CA4 pyramidal neurons of the hippocampus, granular cells and Purkinje neurons of the cerebellum [152, 293, 354]. However, KCC2 protein was detected in dorsal root ganglion neurons and KCC2 mRNA was demonstrated to be present at very low levels in VSMCs, suggesting that KCC2 expression may not be strictly confined to the central nervous system neurons [93, 238]. Murine KCC2 is also expressed in the ventral horns of the spinal cord at embryonic day 12.5 and throughout the spinal cord at birth [156]. The KCC2 protein appears to be localized at inhibitory synapses of the spinal cord [236]. KCC2 is also expressed in inter-neurons in all areas of the rat hippocampus [138], and in some areas of the hypothalamus [224]. KCC2 mRNA was detected in most neurons, Purkinje cells, granule cells, and Stella/basket cells of the rat cerebellum [246]. Expression of KCC2 in non-neuronal tissues is undetectable or extremely low [41] [93, 152].

KCC2 appears to play a crucial role in promoting synaptic inhibition, in controlling central nervous system excitability, and in inhibition of postsynaptic potential [156, 302, 304, 347, 365]. The KCC2 knockout mice have severe motor deficits, exhibit frequent generalized seizures, and die immediately after birth [156, 365]. Animals with heterozygote KCC2 deletion are more prone to epileptic seizures and possess increased resistance to anticonvulsant drugs [365]. Moreover, using quantitative PCR, KCC2 mRNA levels were correlated with increased seizure incidence [302]. Brain-derived neurotrophic factor or neurotrophin-4 induces down-regulation of KCC2 mRNA with the consequent impairment in neuronal Cl extrusion capacity, suggesting a role for KCC2 in the induction and establishment of epileptic activity [303]. Interestingly, unknown mechanisms modulate KCC2 expression after nerve injury in the spinal cord. Indeed, local blockade or knock-down of the spinal KCC2 in intact rats markedly increase sensitivity to pain [69]. Recent reports suggest that hKCC2 has the potential to mediate $NH4^+$ uptake in cultured rat brain neurons [235] and possibly contributes to anoxic/ischemic white matter injury [241]. Tyrosine kinase pathways stimulated by insulin and IGF-1 rapidly activate KCC2 in cultured neurons at either the membrane level or by trafficking new KCC2 molecules to the membrane from vesicular pools [188].

KCC3

A close look at the Human Genome Database combined with *in silico* analysis reveals that the human KCC3 gene is located on the reverse strand of locus SLC12A6 in chromosome 15 (cytogenetic marker 15q13) extending from base 28730345 to base 28702321 and with the potential to generate at least 3 different isoforms, KCC3a, KCC3b, and KCC3c. The predicted hKCC3a and KCC3b proteins contain 12 membrane-spanning segments and 5 potential N-glycosylation sites. Both KCC3s share around 77% amino-acid identity with hKCC1 and 73% identity with hKCC2. Several KCC3 mRNA transcripts (9, 7.5, and 4.5 kb) were detected by Northern blot analysis in brain, heart, skeletal muscle, and kidney. However, Western blot analysis only showed expression of 150 kD KCC3 protein that was reduced to 120 kD after glycosidase treatment [151]. Mount et al. identified a full-length hKCC cDNA, which they initially termed KCC4 but later renamed KCC3 [251]. This cDNA encodes an 1150 amino acid protein. Northern blot analysis detected 2 KCC3 transcripts of 6–7 kb, consistent with alternative splicing, in muscle, brain, lung, heart, and kidney. The longer KCC3 isoform was designated as KCC3a [251] and the shorter isoform KCC3b [151].

At least four different hKCC3 cDNAs were described (AF477977, AF105366, AF116242, and AF108131) altogether differing at their 5′-UTR. Three human cDNAs differing at their 5′-UTR encode for the KCC3a isoform (AF116242, and AF105366), whereas KCC3b is encoded by a single cDNA (Fig. 14). The molecular mechanism, as well as the genomic DNA sequences, involved in the transcription of different 5′-UTR KCC3a mRNAs are presently unknown. In all KCC3a mRNAs, a single exon (exon 1a) encodes for the first 92 amino acids. Exon 1a is located 23 kb upstream from exon 1b. Then, all KCC3a transcripts appear to be the result of selection of an alternative promoter 5′ exon 1a as well as an alternative splicing, since exon 1b and part of exon 2 (the first 21 bp) are not present in all KCC3a mRNA isoforms.

The hKCC3 gene seems to produce the KCC3b mRNA isoform by direct transcription of 22 confirmed exons that follow the consensual gt-ag splicing rule. However, the DNA sequence of the promoter region upstream of exon Ib is still incomplete. Two identical cloned cDNAs encode for the KCC3b isoform (NM_005135 and AF105366) [151, 251]. The first 32 residues in the KCC3b protein are encoded by a single exon (exon 1b) separated from exon 2 by almost 2 kb of intronic sequence. The second exon in the hKCC3 gene encodes for the next 44 residues. The first 7 amino acids encoded in exon 2 are only present in KCC3b, whereas the rest of the C-terminal encoded amino acids are common to all KCC3 iso-

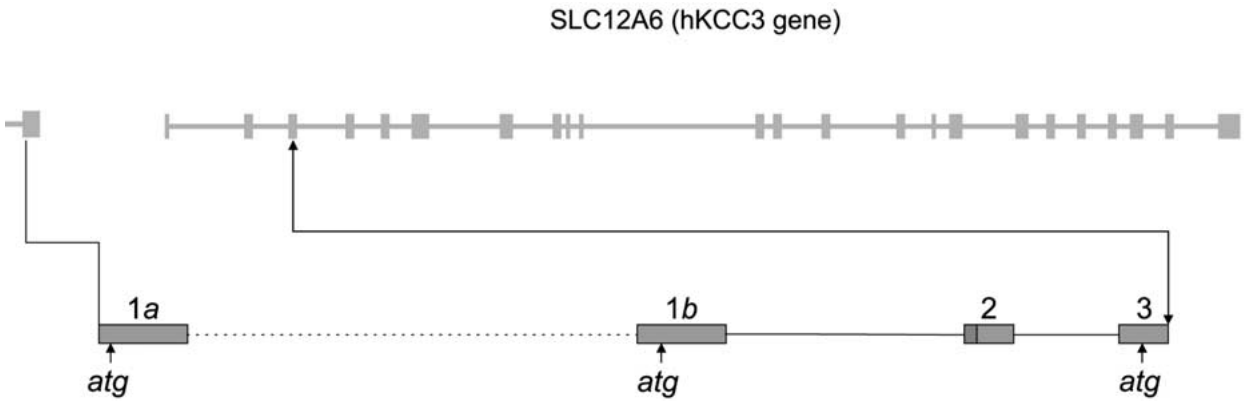

Fig. 14. Genomic organization of hKCC3 gene. Representative diagram of the genomic organization of human KCC3 gene (SLC12A6), in which all the coding exons are indicated as grey boxes connected by their respective introns.

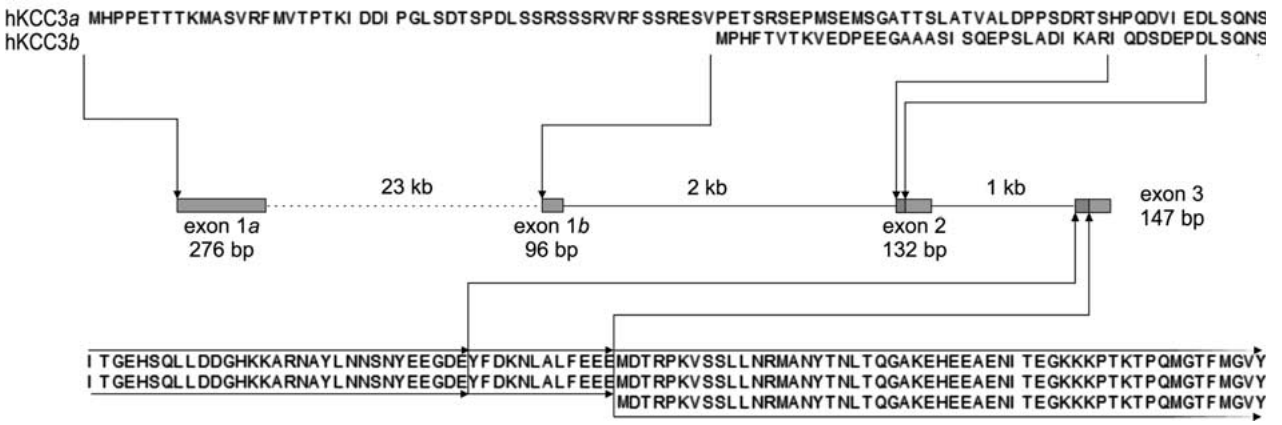

Fig. 15. Alternative initiation codons in the hKCC3 gene. Representative not-to-scale diagram of the 5′ end of hKCC3 gene showing three alternative start codons located at exon 1a, exon 1b, and exon 3, respectively. Exon 1b encodes for the unique first 33 amino acids of KCC3b, whereas exon 2 encodes for the last 8 unique amino acids of KCC3b. Exon 2 also shows the relative position of the predicted splicing acceptor site for exon 1b (24 bp 3′ exon 2). The N-terminal regions of all KCC3 proteins are aligned, showing the relative initiation position.

forms. KCC3b mRNA encodes a protein of 1099 amino acids, with a predicted molecular weight of 122 kD, and different content and distribution of potential phosphorylation sites.

A more complex picture arises by the report of a new cDNA (AF477977) with 774 bp of 5′-UTR, which here we call KCC3c, the shortest KCC3 version (1012 amino acids) with a predicted molecular weight of 112 kD. *In silico* analysis of KCC3c mRNA predicts translation from an alternative in-frame initiation codon (ATG) located in exon 3, probably because exon 1b and 2 were spliced out at the pre-mRNA level (Fig. 15). A similar, but not identical full-length cDNA clone with the potential to encode for at least two truncated versions of hKCC3 was also described (BC033894). Although its significance and tissue distribution is unknown, a different version of KCC3 with an approximate apparent molecular weight of 105–110 kD was detected in the mouse kidney [294]. The difference between this short KCC3 version and KCC3a expressed in mouse brain remained after deglycosylation, indicating that the difference is not related to differential glycosylation [294].

KCC3 transcripts were detected in human kidney, heart, and brain [151, 251]. Although little is known about the functional properties of the KCC3 gene product, a recent report suggests that KCC3 may play an important role in the regulation of cell growth [326–328, 373]. KCC3 was suggested to play a role in the physiology of myelinization since the ontogeny of KCC3 expression correlates with myelinization in the rodent central nervous system [294]. Furthermore, KCC3 is mutant in a severe peripheral neuropathy associated with agenesis of the corpus callosum [155]. Mutations in all 25 exons in the KCC3 gene were screened in an attempt to correlate hKCC3 anomalies with rolandic epilepsy or common subtypes of idiopathic generalized epilepsy. However, no significant correlations were found [338].

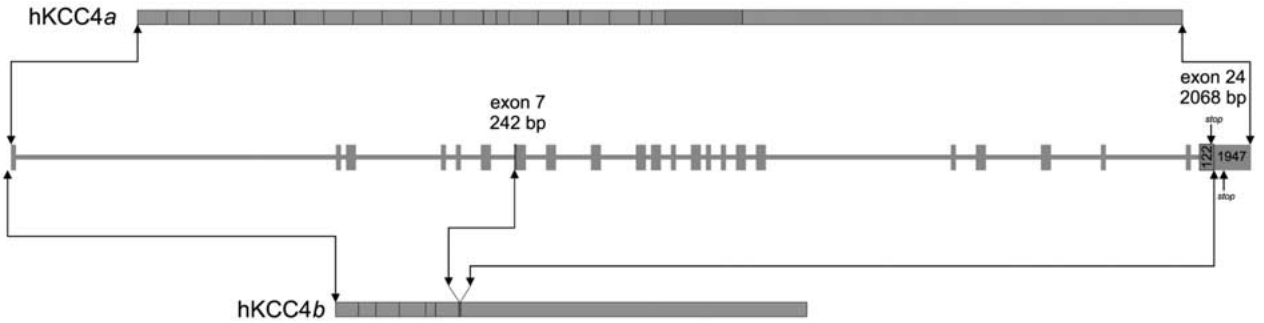

Fig. 16. Genomic organization of the hKCC4 gene. Representative diagram of the genomic organization of the KCC4 gene. The gene is defined by 24 exons (light gray boxes), which have the potential to generate 2 variants: KCC4a and KCC4b (indicated as continuous light gray boxes).

The pattern of KCC3a and KCC3b expression in some mouse tissues and in rat VSMCs was recently analyzed by Northern blot and RT-PCR, respectively [89, 294]. Although the precise mechanisms involved in the production of several KCC3a mRNAs and the KCC3b mRNA are unknown, the presence of several 5′-UTR unique KCC3 mRNAs, and their N-terminal KCCSs raise the possibilities of differential transcriptional, post-transcriptional, and/or post-translational regulation. Supporting the hypothesis of post-transcriptional regulation is the fact that both KCC3a and KCC3b mRNAs are differentially upregulated by the NO signaling pathway independently of de novo transcription [8, 90, 92, 93]. The relevance of NO per se and fast NO releasers in the upregulation of KCC3 mRNA in rat VSMCs was recently determined [92]. In line with these findings, KCC3 knockout mice are severely hypertensive [2]. This effect remains to be investigated in the human KCC3 mutation.

KCC4

The fourth version of human genes for KCCs, hKCC4, is encoded by 24 exons located in locus SLC12A7 in a genomic region spanning 60 kb on human chromosome 5 (5p15.3, sequence NT_006576). At least three full-length human cDNAs were characterized so far, two long versions (XM_049508, and NM_006598) encoding for an identical KCC4a protein of 1083 amino acids [251], and a short version obtained from brain neuroblastoma cells (BC007760) encoding for KCC4b, an alternatively spliced isoform, which encodes for a protein of 266 amino acids (MW 28 kD) (Fig. 16). Although probably a non-active isoform, *in silico* analysis suggests that KCC4b is the result of either a homozygous gene deletion or an alternative splicing, since 2063 bp corresponding to the last 240 bp of exon 7, together with exons 8 to 22, and the first 122 bp of the last exon, are absent (Fig. 16). KCC4a and KCC4b proteins share an identical cytoplasmic N-terminal domain and the first 3 transmembrane domains. The extracellular C-terminal domain present in KCC4b is encoded by the first 2 bp of exon 7 and 126 nucleotides located in the last 1947 bp of exon 23 (Fig. 16). The physiological significance, of KCC4b is unknown.

The hKCC4a protein is 90 % and 71 % homologous to the mouse and rat counterparts, respectively, hKCC4a mRNA is a 5.3 kb transcript that is expressed in bone marrow, spleen, thymus, brain, spinal cord, skeletal muscle, prostate, pancreas, and basolateral membranes of several nephron segments, lung, heart, and liver. It was postulated that KCC4 mediates potassium and chloride exit from the cell and may play an important role in salt absorption by the distal convoluted tubule [350]. A recent report describes in detail the distribution of KCC4a protein in the central nervous system and shows that it is highly expressed in cranial nerves, spinal cord and peripheral nerves [183]. Molecular disruption of mouse KCC4 gene expression is associated with deafness and renal tubular acidosis. Although viable and fertile, the KCC4 knockout mice weigh about 90% of their littermates and their hearing ability quickly deteriorates becoming almost totally deaf after the second week of life [33]. The KCC4 gene appears to be implicated neither in the normal development of the inner ear nor in endolymph production, since no obvious histological changes and no collapse of the Reissner membrane were found. However, at the cochlear level, the outer hair cells of basal turns were absent and the organ of Corti was lost completely in basal turns [33].

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References

1. Adragna, N.C., Ahmed, H., Ferrell, C.M., Filosa, J., Lauf, P.K. 1999. New mechanism of regulation of K-Cl cotransport. *Biophys. J.* **76:**A393

2. Adragna, N.C., Chen, Y., Delpire, E., Lauf, P.K., Morris, M. 2004. Hypertension in K-Cl cotransporter-3 knockout mice. *In:* 3rd. Internat. Symp. Cell Volume Regulation and Signaling. Lauf, P.K., Adragna, N.C, (eds)., pp 379–386. Kluwer/Plenum Press, Dayton, OH
3. Adragna, N.C., Di Fulvio, M., Ferrell, C.M., Lauf, P.K. 2000. Lipid-protein interactions and signal transduction. *Abstract Book, First Mercosur Workshop on Biomembranes*. :29–30
4. Adragna, N.C., Lauf, P.K. 1994. Quinine and quinidine inhibit and reveal heterogeneity of K-Cl cotransport in low K sheep erythrocytes. *J. Membrane Biol.* **142:**195–207
5. Adragna, N.C., Lauf, P.K. 1997. Oxidative activation of K-Cl cotransport by diamide in erythrocytes from humans with red cell disorders, and from several other mammalian species. *J. Membrane Biol.* **155:**207–217
6. Adragna, N.C., Lauf, P.K. 1998. Role of nitrite, a nitric oxide derivative, in K-Cl cotransport activation of low-potassium sheep red blood cells. *J. Membrane Biol.* **166:**157–167
7. Adragna, N.C., White, R.E., Orlov, S.N., Lauf, P.K. 2000. K-Cl cotransport in vascular smooth muscle and erythrocytes: possible implication in vasodilation. *Am. J.* **278:**C381–390
8. Adragna, N.C., Zhang, J., Di Fulvio, M., Lincoln, T.M., Lauf, P.K. 2002. K-Cl cotransport regulation and protein kinase G in cultured vascular smooth muscle cells. *J. Membrane Biol.* **187:**157–165
9. Agalakova, N.I., Lapin, A.V., Gusev, G.P. 1997. Differential effects of glycolytic and oxidative metabolism blockers on the Na-K pump in erythrocytes of the frog, *Rana temporaria*. *J. Comp. Physiol. [B]*. **167:**576–581
10. Agalakova, N.I., Lapin, A.V., Gusev, G.P. 1997. Temperature effects on ion transport across the erythrocyte membrane of the frog *Rana temporaria*. *Comp. Biochem. Physiol. A Physiol.* **117:**411–418
11. Agalakova, N.I., Lapin, A.V., Gusev, G.P. 2000. Effects of fluoride and vanadate on K^+ transport across the erythrocyte membrane of *Rana temporaria*. *Membr. Cell Biol.* **13:**527–536
12. Aiton, J.F., Simmons, N.L. 1984. An effect of piretanide upon the intracellular cation contents of cells subjected to partial chronic (Na-K) pump blockade by ouabain. *Biochem. Pharmacol.* **33:**3425–3431
13. Alabovskii, V.V., Cragoe, E.J. Jr., Vinokurov, A.A. 1999. The role of ion transport system of sarcolemma in amino acid loss during myocardial perfusion with calcium-free medium and myocardium injury during "calcium paradox". *Vopr. Med. Khim.* **45:**238–245
14. Amorim, J.B., Bailey, M.A., Musa-Aziz, R., Giebisch, G., Malnic, G. 2003. Role of luminal anion and pH in distal tubule potassium secretion. *Am. J. Physiol.* **284:**F381–388
15. Anagnostopoulos, T., Edelman, A., Planelles, G., Teulon, J., Thomas, S.R. 1984. Transport of chlorine in the proximal tubule. Its effects on water-electrolyte absorption. *J. Physiol.* **79:**132–138
16. Andersen, G.O., Enger, M., Thoresen, G.H., Skomedal, T., Osnes, J.B. 1998. Alphal-adrenergic activation of myocardial Na-K-2Cl cotransport involving mitogen-activated protein kinase. *Am. J. Physiol.* **275:**H641–652
17. Anfinogenova, Y.J., Rodriguez, X., Grygorczyk, R., Adragna, N.C., Lauf, P.K., Hamet, P., Orlov, S.N. 2001. Swelling-induced K^+ fluxes in vascular smooth muscle cells are mediated by charybdotoxin-sensitive K^+ channels. *Cell Physiol. Biochem.* **11:**295–310
18. Armsby, C.C., Brugnara, C., Alper, S.L. 1995. Cation transport in mouse erythrocytes: role of K^+-Cl^- cotransport in regulatory volume decrease. *Am. J. Physiol.* **268:**C894–902
19. Armsby, C.C., Stuart-Tilley, A.K., Alper, S.L., Brugnara, C. 1996. Resistance to osmotic lysis in BXD-31 mouse erythrocytes: association with upregulated K-Cl cotransport. *Am. J. Physiol.* **270:**C866–877
20. Baldisserotto, B., Mimura, O.M. 1994. Ion transport across the isolated intestinal mucosa of *Anguilla anguilla* (Pisces). *Comp. Biochem. Physiol. Comp. Physiol.* **108:**297–302
21. Ballanyi, K., Grafe, P., Reddy, M.M., ten Bruggencate, G. 1984. Different types of potassium transport linked to carbachol and gamma-aminobutyric acid actions in rat sympathetic neurons. *Neuroscience*. **12:**917–927
22. Bergeron, M.J., Gagnon, E., Wallendorff, B., Lapointe, J.Y., Isenring, P. 2003. Ammonium transport and pH regulation by cotransporters. *Am. J. Physiol.* **285:**F68–F78
23. Bergh, C., Kelley, S.J., Dunham, P.B. 1990. K-Cl cotransport in LK sheep erythrocytes: kinetics of stimulation by cell swelling. *J. Membrane Biol.* **117:**177–188
24. Bernhardt, I., Erdmann, A., Vogel, R., Glaser, R. 1987. Factors involved in the increase of K^+ efflux of erythrocytes in low chloride media. *Biomed. Biochim. Acta*. **46:**S36–S40
25. Bianchini, L., Fossat, B., Porthe-Nibelle, J., Ellory, J.C., Lahlou, B. 1988. Effects of hyposmotic shock on ion fluxes in isolated trout hepatocytes. *J. Exp. Biol.* **137:**303–318
26. Bize, I. 2001. Theoretical validation for a model of KCC regulation in human erythrocytes. *Blood Cells Mol Dis.* **27:**121–126
27. Bize, L., Dunham, P.B. 1994. Staurosporine, a protein kinase inhibitor, activates K-Cl cotransport in LK sheep erythrocytes. *Am. J. Physiol.* **266:**C759–C770
28. Bize, L., Dunham, P.B. 1995. H_2O_2 activates red blood cell K-Cl cotransport via stimulation of a phosphatase. *Am. J. Physiol.* **269:**C849–C855
29. Bize, L., Guvenc, B., Buchbinder, G., Brugnara, C. 2000. Stimulation of human erythrocyte K-Cl cotransport and protein phosphatase type 2A by n-ethylmaleimide: role of intracellular Mg^{++}. *J. Membrane Biol.* **177:**159–168
30. Bize, L., Guvenc, B., Robb, A., Buchbinder, G., Brugnara, C. 1999. Serine/threonine protein phosphatases and regulation of K-Cl cotransport in human erythrocytes. *Am. J. Physiol.* **277:**C926–C936
31. Blum, J.J. 1992. Effect of osmolality on $86Rb^+$ uptake and release by Leishmania donovani. *Cell Physiol.* **152:**111–117
32. Boerth, N.J., Dey, N.B., Cornwell, T.L., Lincoln, T.M. 1997. Cyclic GMP-dependent protein kinase regulates vascular smooth muscle cell phenotype. *J. Vasc. Res.* **34:**245–259
33. Boettger, T., Hubner, C.A., Maier, H., Rust, M.B., Beck, F.X., Jentsch, T.J. 2002. Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter KCC4. *Nature*. **416:**874–878
34. Bogdanova, A.Y., Gassmann, M., Nikinmaa, M. 2002. Copper ion redox state is critical for its effects on ion transport pathways and methaemoglobin formation in trout erythrocytes. *Chem. Biol. Interact.* **139:**43–59
35. Bogdanova, A.Y., Nikinmaa, M. 2001. Reactive oxygen species regulate oxygen-sensitive potassium flux in rainbow trout erythrocytes. *J. Gen. Physiol.* **117:**181–190
36. Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J., Cohen, R.A. 1994. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature*. **368:**850–853
37. Bookchin, R.M., Lew, V.L. 2002. Sickle red cell dehydration: mechanisms and interventions. *Curr. Opin. Hematol.* **9:**107–110
38. Bookchin, R.M., Ortiz, O.E., Lew, V.L. 1991. Evidence for a direct reticulocyte origin of dense red cells in sickle cell anemia. *J. Clin. Invest.* **87:**113–124

39. Bourgeois, S., Masse, S., Paillard, M., Houillier, P. 2002. Basolateral membrane Cl^-, Na^+-, and K^+-coupled base transport mechanisms in rat MTALH. *Am. J. Physiol.* **282:**F655–F668
40. Bowles, D.W., Gillen, C.M. 2001. Characterization of Rb uptake into Sf9 cells using cation chromatography: evidence for a K-Cl cotransporter. *J. Insect Physiol.* **47:**523–532
41. Brauer, M., Frei, E., Claes, L., Grissmer, S., Jager, H. 2003. Influence of K-Cl cotransporter activity on activation of volume-sensitive Cl-channels in human osteoblasts. *Am. J. Physiol.* **285:**C22–C30
42. Breitwieser, G.E., Altamirano, A.A., Russell, J.M. 1996. Elevated $[Cl^-]_i$, and $[Na^+]_i$ inhibit Na^+, K^+, Cl^- cotransport by different mechanisms in squid giant axons. *J. Gen. Physiol.* **107:**261–270
43. Brock, T.A., Brugnara, C., Canessa, M., Gimbrone, M.A. Jr. 1986. Bradykinin and vasopressin stimulate Na^+-K^+-Cl^- cotransport in cultured endothelial cells. *Am. J. Physiol.* **250:**C888–895
44. Brown, C.D., Murer, H. 1985. Characterization of a Na : K : 2Cl cotransport system in the apical membrane of a renal epithelial cell line (LLC-PK1). *J. Membrane Biol.* **87:**131–139
45. Brugnara, C. 1993. Membrane transport of Na and K and cell dehydration in sickle erythrocytes. *Experientia.* **49:**100–109
46. Brugnara, C. 1995. Erythrocyte dehydration in pathophysiology and treatment of sickle cell disease. *Curr. Opin. Hematol.* **2:**132–138
47. Brugnara, C. 1997. Erythrocyte membrane transport physiology. *Curr. Opin. Hematol.* **4:**122–127
48. Brugnara, C. 2001. Therapeutic strategies for prevention of sickle cell dehydration. *Blood Cells Mol. Dis.* **27:**71–80
49. Brugnara, C., Churchill, W.H. 1992. Effect of irradiation on red cell cation content and transport. *Transfusion.* **32:**246–252
50. Brugnara, C., de Franceschi, L. 1993. Effect of cell age and phenylhydrazine on the cation transport properties of rabbit erythrocytes. *J. Cell Physiol.* **154:**271–280
51. Brugnara, C., Kruskall, M.S., Johnstone, R.M. 1993. Membrane properties of erythrocytes in subjects undergoing multiple blood donations with or without recombinant erythropoietin. *Br. J. Haematol.* **84:**118–130
52. Brugnara, C., Van Ha, T., Tosteson, D.C. 1989. Role of chloride in potassium transport through a K-Cl cotransport system in human red blood cells. *Am. J. Physiol.* **256:**C994–C1003
53. Campbell, E.H., Cossins, A.R., Gibson, J.S. 1999. Oxygen-dependent K^+ influxes in Mg^{2+}-clamped equine red blood cells. *J. Physiol.* **515:**431–437
54. Canessa, M. 1991. Red cell volume-related ion transport systems in hemoglobinopathies. *Hematol. Oncol. Clin. North Am.* **5:**495–516
55. Canessa, M., Fabry, M.E., Blumenfeld, N., Nagel, R.L. 1987. Volume-stimulated, Cl^- dependent K^+ efflux is highly expressed in young human red cells containing normal hemoglobin or HbS. *J. Membrane Biol.* **97:**97–105
56. Canessa, M., Fabry, M.E., Nagel, R.L. 1987. Deoxygenation inhibits the volume-stimulated, Cl^--dependent K^+ efflux in SS and young AA cells: a cytosolic Mg^{2+} modulation. *Blood.* **70:**1861–1866
57. Canessa, M., Fabry, M.E., Suzuka, S.M., Morgan, K., Nagel, R.L. 1990. Na^+ H^+ exchange is increased in sickle cell anemia and young normal red cells. *J. Membrane Biol.* **116:**107–115
58. Canessa, M., Romero, J.R., Lawrence, C., Nagel, R.L., Fabry, M.E. 1994. Rate of activation and deactivation of K:Cl cotransport by changes in cell volume in hemoglobin SS, CC and AA red cells. *Membrane Biol.* **142:**349–362
59. Canessa, M., Spalvins, A., Nagel, R.L. 1986. Volume-dependent and NEM-stimulated K^+, Cl^- transport is elevated in oxygenated SS, SC and CC human red cells. *FEBS Lett.* **200:**197–202
60. Casula, S., Shmukler, B.E., Wilhelm, S., Stuart-Tilley, A.K., Su, W., Chernova, M.N., Brugnara, C., Alper, S.L. 2001. A dominant negative mutant of the KCC1 K-Cl cotransporter: both N- and C-terminal cytoplasmic domains are required for K-Cl cotransport activity. *J. Biol. Chem.* **276:**41870–41878
61. Chang, H., Fujita, T. 1999. A numerical model of the renal distal tubule. *Am. J. Physiol.* **276:**F931–F951
62. Chen, P.Y., Verkman, A.S. 1988. Sodium-dependent chloride transport in basolateral membrane vesicles isolated from rabbit proximal tubule. *Biochemistry.* **27:**655–660
63. Chipperfield, A.R. 1985. Loop diuretics may fail to inhibit (Na^+, K^+ Cl^-) 'cotransport' in human red cells. *Biosci. Rep.* **5:**299–302
64. Chipperfield, A.R., Harper, A.A. 2000. Chloride in smooth muscle. *Prog. Biophys. Mol. Biol.* **74:**175–221
65. Colclasure, G.C., Parker, J.C. 1992. Cytosolic protein concentration is the primary volume signal for swelling-induced [K-Cl] cotransport in dog red cells. *J. Gen. Physiol.* **100:**1–10
66. Colclasure, G.C., Parker, J.C. 1993. ATP dependence of K-Cl cotransport in dog red blood cells. *Am. J. Physiol.* **265:**C1648–C1652
67. Colclasure, G.C., Parker, J.C., Dunham, P.B. 1995. Creatine kinase is required for swelling-activated K-Cl cotransport in dog red blood cells. *Am. J. Physiol.* **268:**C660–C668
68. Cossins, A.R., Gibson, J.S. 1997. Volume-sensitive transport systems and volume homeostasis in vertebrate red blood cells. *J. Exp. Biol.* **200:**343–352
69. Coull, J.A., Boudreau, D., Bachand, K., Prescott, S.A., Nault, F., Sik, A., De Koninck, P., De Koninck, Y. 2003. Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature.* **424:**938–942
70. Crook, R.B., Riese, K. 1996. Beta-adrenergic stimulation of $Na^+/K^+/Cl^-$ cotransport in fetal nonpigmented ciliary epithelial cells. *Invest. Ophthalmol Vis. Sci.* **37:**1047–1057
71. Crook, R.B., von Brauchitsch, D.K., Polansky, J.R. 1992. Potassium transport in nonpigmented epithelial cells of ocular ciliary body: inhibition of a Na^+, K^+, Cl^- cotransporter by protein kinase C. *J. Cell Physiol.* **153:**214–220
72. De Franceschi, L., Bachir, D., Galacteros, F., Tchernia, G., Cynober, T., Alper, S., Platt, O., Beuzard, Y., Brugnara, C. 1997. Oral magnesium supplements reduce erythrocyte dehydration in patients with sickle cell disease. *J. Clin. Invest.* **100:**1847–1852
73. De Franceschi, L., Beuzard, Y., Brugnara, C. 1995. Sulfhydryl oxidation and activation of red cell K^+-Cl^- cotransport in the transgenic SAD mouse. *Am. J. Physiol.* **269:**C899–906
74. De Franceschi, L., Brugnara, C., Beuzard, Y. 1997. Dietary magnesium supplementation ameliorates anemia in a mouse model of beta-thalassemia. *Blood.* **90:**1283–1290
75. De Franceschi, L., Fattovich, G., Turrini, F., Ayi, K., Brugnara, C., Manzato, F., Noventa, F., Stanzial, A.M., Solero, P., Corrocher, R. 2000. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. *Hepatology.* **31:**997–1004
76. De Franceschi, L., Fumagalli, L., Olivieri, O., Corrocher, R., Lowell, C.A., Berton, G. 1997. Deficiency of Src family kinases Fgr and Hck results in activation of erythrocyte K/Cl cotransport. *J. Clin. Invest.* **99:**220–227
77. De Franceschi, L., Olivieri, O., Miraglia del Giudice, E., Perrotta, S., Sabato, V., Corrocher, R., Iolascon, A. 1997. Membrane cation and anion transport activities in erythro-

cytes of hereditary spherocytosis: effects of different membrane protein defects. *Am. J. Hematol.* **55:**121–128
78. De Franceschi, L., Villa-Moruzzi, E., Fumagalli, L., Brugnara, C., Turrini, F., Motta, R., Veghini, E., Corato, C., Alper, S.L., Berton, G. 2001. K-Cl cotransport modulation by intracellular Mg in erythrocytes from mice bred for low and high Mg levels. *Am. J. Physiol.* **281:**C1385–C1395
79. De Giorgi, A., Carnimeo, L., Corcelli, A. 1992. Mechanism of Cl-transport in eel intestinal brush-border membrane vesicles. *Pftuegers Arch.* **420:**551–558
80. de Silva, H.A., Aronson, J.K. 1997. Evidence for an R(+) [(dihydroindenyl)oxy]alkanoic acid-sensitive K^+/Cl^- co-transporter in human platelets and its interaction with the $Na^+/K^+/2Cl^-$ co-transporter. *Clin. Sci. (Lond).* **93:**243–248
81. DeFazio, R.A., Keros, S., Quick, M.W., Hablitz, J.J. 2000. Potassium-coupled chloride cotransport controls intracellular chloride in rat neocortical pyramidal neurons. *J. Neurosci.* **20:**8069–8076
82. Delpire, E. 2000. Cation-chloride, cotransporters in neuronal communication. *News Physiol. Sci.* **15:**309–312
83. Delpire, E., Gullans, S.R. 1994. Cell volume and K^+ transport during differentiation of mouse erythroleukemia cells. *Am. J. Physiol.* **266:**C515–C523
84. Delpire, E., Lauf, P.K. 1991. Kinetics of Cl-dependent K fluxes in hyposmotically swollen low K sheep erythrocytes. *J. Gen. Physiol.* **97:**173–193
85. Delpire, E., Lauf, P.K. 1991. Trans effects of cellular K and Cl on ouabain-resistant Rb(K) influx in low K sheep red blood cells: further evidence for asymmetry of K-Cl cotransport. *Pfluegers Arch.* **419:**540–542
86. Delpire, E., Lauf, P.K. 1991. Magnesium and ATP dependence of K-Cl co-transport in low K^+ sheep red blood cells. *J. Physiol.* **441:**219–231
87. Delpire, E., Lauf, P.K. 1992. Kinetics of DIDS inhibition of swelling-activated K-Cl cotransport in low K sheep erythrocytes. *J. Membrane Biol.* **126:**89–968
88. Delpire, E., Mount, D.B. 2002. Human and murine phenotypes associated with defects in cation-chloride cotransport. *Annu. Rev. Physiol.* **64:**803–843
89. Di Fulvio, M., Lauf, P.K., Adragna, N.C. 2001. Nitric oxide signaling pathway regulates potassium chloride cotransporter-1 mRNA expression in vascular smooth muscle cells. *J. Biol. Chem.* **276:**44534–44540
90. Di Fulvio, M., Lauf, P.K., Adragna, N.C. 2002. Protein kinase G (PKG) signaling pathway regulates the potassium-chloride cotransporter-3 (KCC3a, and KCC3b) mRNA expression in primary cultures of rat vascular smooth muscle cells (VSMCs). *FASEB J.* **16:**A421
91. Di Fulvio, M., Lauf, P.K., Adragna, N.C. 2003. The NO signaling pathway differentially regulates KCC3a and KCC3b mRNA expression. *Nitric Oxide.* **9:**165–171
92. Di Fulvio, M., Lauf, P.K., Shah, S., Adragna, N.C. 2003. NONOates regulate potassium-chloride cotransporter-1 and -3 mRNA expression in vascular smooth muscle cells. *Am. J. Physiol.* **284:**H1686–H1692
93. Di Fulvio, M., Lincoln, T.M., Lauf, P.K., Adragna, N.C. 2001. Protein kinase G regulates potassium chloride cotransporter-3 expression in primary cultures of rat vascular smooth muscle cells. *J. Biol Chem.* **276:**21046–21052
94. Diecke, F.P., Beyer-Mears, A. 1997. A mechanism for regulatory volume decrease in cultured lens epithelial cells. *Curr. Eye Res.* **16:**279–88
95. Duhm, J., Heller, J., Zicha, J. 1990. Kinetics of red cell Na^+ and K^+ transport in Prague hypertensive rats. *Clin. Exp. Hypertens. A.* **12:**1203–1222
96. Dunham, P.B. 1992. Ion transport in sheep red blood cells. *Comp. Biochem. Physiol. Comp. Physiol.* **102:**625–630
97. Dunham, P.B. 1995. Effects of urea on K-Cl cotransport in sheep red blood cells: evidence for two signals of swelling. *Am. J. Physiol.* **268:**C1026–C1032
98. Dunham, P.B., Blostein, R. 1997. L antigens of sheep red blood cell membranes and modulation of ion transport. *Am. J. Physiol.* **272:**C357–C368
99. Dunham, P.B., Klimczak, J., Logue, P.J. 1993. Swelling activation of K-Cl cotransport in LK sheep erythrocytes: a three-state process. *J. Gen. Physiol.* **101:**733–765
100. Dunham, P.B., Logue, P.J. 1986. Potassium-chloride cotransport in resealed human red cell ghosts. *Am. J. Physiol.* **250:**C578–583
101. Dunham, P.B., Stewart, G.W., Ellory, J.C. 1980. Chloride-activated passive potassium transport in human erythrocytes. *Proc. Natl. Acad. Sci. USA.* **77:**1711–1715
102. Ellison, D.H., Velazquez, H., Wright, F.S. 1985. Stimulation of distal potassium secretion by low lumen chloride in the presence of barium. *Am. J. Physiol.* **248:**F638–F649
103. Ellory, J.C., Gibson, J.S., Stewart, G.W. 1998. Pathophysiology of abnormal cell volume in human red cells. *Contrib. Nephrol.* **123:**220–239
104. Etzion, Z., Lew, V.L., Bookchin, R.M. 1996. K (86Rb) transport heterogeneity in the low-density fraction of sickle cell anemia red blood cells. *Am. I. Physiol.* **271:**C1111–C1121
105. Eveloff, J., Kinne, R. 1983. Sodium-chloride transport in the medullary thick ascending limb of Henle's loop: evidence for a sodium-chloride cotransport system in plasma membrane vesicles. *J. Membrane Biol.* **2:**173–181
106. Eveloff, J., Warnock, D.G. 1987. K-Cl transport systems in rabbit renal basolateral membrane vesicles. *Am. I. Physiol.* **252:**F883–F889
107. Eveloff, J.L., Warnock, D.G. 1987. Activation of ion transport systems during cell volume regulation. *Am. J. Physiol.* **252:**F1–F10
108. Fabry, M.E., Kaul, D.K. 1991. Sickle cell vaso-occlusion. *Hematol. Oncol. Clin. North Am.* **5:**375–398
109. Fabry, M.E., Romero, J.R., Buchanan, I.D., Suzuka, S.M., Stamatoyannopoulos, G., Nagel, R.L., Canessa, M. 1991. Rapid increase in red blood cell density driven by K:Cl cotransport in a subset of sickle cell anemia reticulocytes and discocytes. *Blood.* **78:**217–225
110. Fabry, M.E., Romero, J.R., Suzuka, S.M., Gilman, J.G., Feeling-Taylor, A., Odunusi, E., Factor, S.M., Bouhassira, E.E., Lawrence, C., Nagel, R.L. 2000. Hemoglobin C in transgenic mice: effect of HbC expression from founders to full mouse globin knockouts. *Blood Cells Mol. Dis.* **26:**331–347
111. Fahraeus, C., Theander, S., Edman, A., Grampp, W. 2002. The K-Cl cotransporter in the lobster stretch receptor neurone—a kinetic analysis. *J. Theor. Biol.* **217:**287–309
112. Feray, J.C., Garay, R. 1988. Demonstration of a Na^+: Mg^{2+} exchange in human red cells by its sensitivity to tricyclic antidepressant drugs. *Naunyn Schmiedebergs Arch. Pharmacol.* **338:**332–337
113. Feray, J.C., Guerrouache, K., Garay, R.P. 2000. Dramatic magnesium efflux induced by high potassium in rat thymocytes. *Biochem. Biophys. Res. Commun.* **268:**673–676
114. Ferrell, C.M., Lauf, P.K., Wilson, B.A., Adragna, N.C. 2000. Lithium and protein kinase C modulators regulate swelling-activated K-Cl cotransport and reveal a complete phosphatidylinositol cycle in low K sheep erythrocytes. *J. Membrane Biol.* **177:**81–93
115. Fitzgerald, E., Djamgoz, M., Dunbar, S. 1996. Maintenance of the K^+ activity gradient in insect muscle compared in

diptera and lepidoptera: contributions of metabolic and exchanger mechanisms. *J. Exp. Biol.* **199:**1857–1872
116. Flatman, P.W., Adragna, N.C., Lauf, P.K. 1996. Role of protein kinases in regulating sheep erythrocyte K-Cl cotransport. *Am. J. Physiol.* **271:**C255–C263
117. Frengen, E., Thomsen, P.D., Brede, G., Solheim, J., de Jong, P.J., Prydz, H. 1997. The gene cluster containing the LCAT gene is conserved between human and pig. *Cytogenet. Cell Genet.* **76:**53–57
118. Fujise, H., Abe, K., Kamimura, M., Ochiai, H. 1997. K^+ Cl^- cotransport and volume regulation in the light and the dense fraction of high-K^+ dog red blood cells. *Am. J. Physiol.* **273:**R991–R998
119. Fujise, H., Higa, K., Kanemaru, T., Fukuda, M., Adragna, N.C., Lauf, P.K. 2001. GSH depletion, K-Cl cotransport, and regulatory volume decrease in high-K/high-GSH dog red blood cells. *Am. J. Physiol.* **281:**C2003–C2009
120. Furlong, T.J., Spring, K.R. 1990. Mechanisms underlying volume regulatory decrease by Necturus gallbladder epithelium. *Am. J. Physiol.* **258:**C1016–C1024
121. Gagnon, F., Dulin, N.O., Tremblay, J., Hamet, P., Orlov, S.N. 1999. ATP-induced inhibition of Na^+, K^+, Cl^- cotransport in Madin-Darby canine kidney cells: lack of involvement of known purinoceptor-coupled signaling pathways. *J. Membrane Biol.* **167:**193–204
122. Ganguly, K., Schinder, A.F., Wong, S.T., Poo, M. 2001. GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell.* **105:**521–532
123. Garay, R.P., Labaune, J.P., Mesangeau, D., Nazaret, C., Imbert, T., Moinet, G. 1990. CRE 10904 [2-(p-fluorophenoxy), 1-(o-hydroxyphenyl)-ethane]: a new diuretic and antihypertensive drug acting by in vivo sulfation. *J. Pharmacol Exp. Ther.* **255:**415–422
124. Garay, R.P., Nazaret, C., Hannaert, P.A., Cragoe, E.J. Jr. 1988. Demonstration of a [K^+, Cl^-]-cotransport system in human red cells by its sensitivity to [(dihydroindenyl)oxy]alkanoic acids: regulation of cell swelling and distinction from the bumetanide-sensitive [Na^+, K^+, Cl^-]-cotransport system. *Mol. Pharmacol.* **33:**696–701
125. Garay, R.P., Rosati, C., Nazaret, C., Esanu, A., Tarrade, T., Braquet, P. 1992. Cicletanine sulfate: inhibition of anion transport systems and natriuretic activity. Naunyn Schmiedebergs. *Arch. Pharmacol.* **346:**114–119
126. Gibson, J.S., Ellory, J.C., Culliford, S.J., Fincham, D.A. 1993. Volume-sensitive KCl co-transport and taurine fluxes in horse red blood cells. *Exp. Physiol.* **78:**685–695
127. Gibson, J.S., Godart, H., Ellory, J.C., Staines, H., Honess, N.A., Cossins, A.R. 1994. Modulation of K^+-Cl^- cotransport in equine red blood cells. *Exp. Physiol.* **79:**997–1009
128. Gibson, J.S., Khan, A., Speake, P.F., Ellory, J.C. 2001. O_2 dependence of K^+ transport in sickle cells: the effect of different cell populations and the substituted benzaldehyde 12C79. *FASEB J.* **15:**823–832
129. Gibson, J.S., Speake, P.F., Ellory, J.C. 1998. Differential oxygen sensitivity of the K^+-Cl^- cotransporter in normal and sickle human red blood cells. *J. Physiol.* **511:**225–234
130. Gibson, J.S., Speake, P.F., Muzyamba, M.C., Husain, F., Luckas, M.C., Ellory, J.C. 2001. K^+ transport in red blood cells from human umbilical cord. *Biochim. Biophys. Acta.* **1512:**231–238
131. Gillen, C.M., Brill, S., Payne, J.A., Forbush, B. 3rd. 1996. Molecular cloning and functional expression of the K-Cl cotransporter from rabbit, rat, and human. A new member of the cation-chloride cotransporter family. *J. Biol Chem.* **271:** 16237–16244
132. Gillen, C.M., Forbush, B. 3rd. 1999. Functional interaction of the K-Cl cotransporter (KCC1) with the Na-K-Cl cotransporter in HEK-293 cells. *Am. J. Physiol.* **276:**C328–C336
133. Goud, C., Di Piero, A., Lockette, W.E., Webb, R.C., Charpie, J.R. 1999. Cyclic GMP-independent mechanisms of nitric oxide-induced vasodilation. *Gen. Pharmacol.* **32:**51–55
134. Grassl, S.M., Holohan, P.D., Ross, C.R. 1987. Cl^--HCO_3 exchange in rat renal basolateral membrane vesicles. *Biochim. Biophys. Acta.* **905:**475–484
135. Grzelak, A., Mazur, J., Bartosz, G. 2001. Peroxynitrite activates K^+-Cl^- cotransport in human erythrocytes. *CellBiol. Int.* **25:**1163–1165
136. Guggino, W.B. 1986. Functional heterogeneity in the early distal tubule of the Amphiuma kidney: evidence for two modes of Cl^- and K^+ transport across the basolateral cell membrane. *Am. J. Physiol.* **250:**F430–F440
137. Guizouarn, H., Motais, R. 1999. Swelling activation of transport pathways in erythrocytes: effects of Cl^-, ionic strength, and volume changes. *Am. J. Physiol.* **276:**C210–C220
138. Gulyas, A.I., Sik, A., Payne, J.A., Kaila, K., Freund, T.F. 2001. The KCl cotransporter, KCC2, is highly expressed in the vicinity of excitatory synapses in the rat hippocampus. *Eur. J. Neurosci.* **13:**2205–2217
139. Gunther, T., Vormann, J. 1989. Na^+-independent Mg^{2+} efflux from Mg^{2+}-loaded human erythrocytes. *FEBS Lett.* **247:**181–184
140. Gusev, G.P., Agalakova, N.I., Lapin, A.V. 1995. Potassium transport in red blood cells of frog *Rana temporaria*, demonstration of a K-Cl cotransport. *J. Comp. Physiol. [B].* **165:**230–237
141. Gusev, G.P., Agalakova, N.I., Lapin, A.V. 1999. Kinetics of K-Cl cotransport in frog erythrocyte membrane: effect of external sodium. *J. Membrane Biol.* **172:**203–213
142. Gusev, G.P., Bogdanova, A. 1992. Evidence for stimulation of the K-Cl cotransport system by phenazine methosulfate. *Biochem. Pharmacol.* **43:**2275–2279
143. Gusev, G.P., Lapin, A.V., Agalakova, N.I. 1999. Inhibition and stimulation of K^+ transport across the frog erythrocyte membrane by furosemide, DIOA, DIDS and quinine. *Gen. Physiol. Biophys.* **18:**269–282
144. Haas, M., Forbush, B. 3rd. 1987. Photolabeling of a 150-kDa (Na^+ K^+ Cl^-) cotransport protein from dog kidney with a bumetanide analogue. *Am. J. Physiol.* **253:**C243–252
145. Haas, M., Forbush, B. 3rd. 1998. The Na-K-Cl cotransporters. *J. Bioenerg Bio. Membrane.* **30:**161–172
146. Haas, M., Forbush, B. 3rd. 2000. The Na-K-Cl cotransporter of secretory epithelia. *Annu. Rev. Physiol.* **62:**515–534
147. Haas, M., Harrison, J.H. Jr. 1989. Stimulation of K-Cl cotransport in rat red cells by a hemolytic anemia- producing metabolite of dapsone. *Am. J. Physiol.* **256:**C265–C272
148. Hall, A.C., Starks, I., Shoults, C.L., Rashidbigi, S. 1996. Pathways for K^+ transport across the bovine articular chondrocyte membrane and their sensitivity to cell volume. *Am. J. Physiol.* **270:**C1300–C1310
149. Hamet, P., Orlov, S.N., Tremblay, J. 1995. Intracellular signaling mechanisms in hypertension. *In:* Laragh, J. H, Brenner, B. M., (editors) Hypertension: Pathophysiology, Diagnosis, and Management. Second Ed. pp 575–607. Raven Press, New York
150. Harper, A.A., Chipperfield, A.R. 1995. Chronotropic action of Na^+, K^+ Cl^- cotransport inhibition in the isolated rat heart. *Eur. J. Pharmacol.* **286:**299–305
151. Hiki, K., D'Andrea, R.J., Furze, J., Crawford, J., Woollatt, E., Sutherland, G.R., Vadas, M.A., Gamble, J.R. 1999. Cloning, characterization, and chromosomal location of a

novel human K^+ Cl^- cotransporter. *J. Biol. Chem.* **274:** 10661–10667
152. Hirosawa, M., Nagase, T., Ishikawa, K., Kikuno, R., Nomura, N., Ohara, O. 1999. Characterization of cDNA clones selected by the GeneMark analysis from size-fractionated cDNA libraries from human brain. *DNA Res.* **6:**329–336
153. Holtzman, E.J., Kumar, S., Faaland, C.A., Warner, F., Logue, P.J., Erickson, S.J., Ricken, G., Waldman, J., Dunham, P.B. 1998. Cloning, characterization, and gene organization of K-Cl cotransporter from pig and human kidney and *C. elegans*. *Am. J. Physiol.* **275:**F550–F564
154. Horiuchi, K., Onyike, A.E., Osterhout, M.L. 1996. Sickling in vitro of reticulocytes from patients with sickle cell disease at venous oxygen tension. *Exp. Hematol.* **24:**68–76
155. Howard, H.C., Mount, D.B., Rochefort, D., Byun, N., Dupre, N., Lu, J., Fan, X., Song, L., Riviere, J.B., Prevost, C., Horst, J., Simonati, A., Lemcke, B., Welch, R., England, R., Zhan, F.Q., Mercado, A., Siesser, W.B., George, A.L. Jr., McDonald, M.P., Bouchard, J.P., Mathieu, J., Delpire, E., Rouleau, G.A. 2002. The K-Cl cotransporter KCC3 is mutant in a severe peripheral neuropathy associated with agenesis of the corpus callosum. *Nat. Genet.* **32:**384–392
156. Hubner, C.A., Stein, V., Hermans-Borgmeyer, L., Meyer, T., Ballanyi, K., Jentsch, T.J. 2001. Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. *Neuron.* **30:**515–524
157. Hume, J.R., Harvey, R.D. 1991. Chloride conductance pathways in heart. *Am. J. Physiol.* **261:**C399–C412
158. Ihrig, L., Hessel, E., Seidler, G., Erdmann, A., Bernhardt, I. 1991. Investigation of monovalent cation influxes of diamide-treated human erythrocytes in solutions of different ionic strength. *Biochim. Biophys. Acta.* **1069:**171–174
159. Ihrig, L., Schonheit, C., Haussner, W., Bernhardt, I. 1992. Characterisation of the potassium influx in rat erythrocytes. *Gen. Physiol. Biophys.* **11:**377–388
160. Ikehara, T., Yamaguchi, H., Hosokawa, K., Takahashi, A., Miyamoto, H. 1993. Kinetic study on the effects of intracellular it K^+ and Na^+ on Na^+, K^+, Cl^- cotransport of HeLa cells by Rb^+ influx determination. *J. Membrane Biol.* **132:**115–124
161. Inagaki, C., Hara, M., Inoue, M. 1992. Neuronal intracellular chloride ion concentrations and their regulatory mechanisms. *Nippon Yakurigaku Zasshi.* **99:**307–315
162. Inagaki, C., Hara, M., Zeng, K.T. 1996. A Cl-pump in rat brain neurons. *J. Exp. Zool.* **275:**262–268
163. Jacob, R., Piwnica-Worms, D., Horres, C.R., Lieberman, M. 1984. Theoretical effects of transmembrane electroneutral exchange on membrane potential. *J. Gen. Physiol.* **83:**47–56
164. Jarolimek, W., Lewen, A., Misgeld, U. 1999. A furosemide-sensitive K^+-Cl^- cotransporter counteracts intracellular Cl^- accumulation and depletion in cultured rat midbrain neurons. *J. Neurosci.* **19:**4695–4704
165. Javaheri, S., Corbett, W., Adams, J.M., Davis, P.J., Gartside, P.S. 1994. Acute respiratory acidosis: large-dose furosemide and cerebrospinal fluid ions. *J. Appl. Physiol.* **76:**2651–2655
166. Javid, P.J., Watts, S.W., Webb, R.C. 1996. Inhibition of nitric oxide-induced vasodilation by gap junction inhibitors: a potential role for cGMP-independent nitric oxide pathway. *J. Vasc. Res.* **33:**395–404
167. Jennings, M.L. 1999. Volume-sensitive K^+/Cl^- cotransport in rabbit erythrocytes. Analysis of the rate-limiting activation and inactivation events. *J. Gen. Physiol.* **114:**743–758
168. Jennings, M.L., Adame, M.F. 2001. Direct estimate of 1:1 stoichiometry of K^+-Cl^- cotransport in rabbit erythrocytes. *Am. J. Physiol. Cell Physiol.* **281:**C825–C832
169. Jennings, M.L., al-Rohil, N. 1990. Kinetics of activation and inactivation of swelling-stimulated K^+/Cl^- transport. The volume-sensitive parameter is the rate constant for inactivation. *J. Gen. Physiol.* **95:**1021–1040
170. Jennings, M.L., Schulz, R.K. 1991. Okadaic acid inhibition of KCl cotransport. Evidence that protein dephosphorylation is necessary for activation of transport by either cell swelling or N-ethylmaleimide. *J. Gen. Physiol.* **97:**799–817
171. Jensen, B.S., Jessen, F., Hoffmann, E.K. 1993. Na^+, K^+, Cl^- cotransport and its regulation in Ehrlich ascites tumor cells. Ca^{2+}/calmodulin and protein kinase C dependent pathways. *J. Membrane Biol.* **131:**161–178
172. Jensen, F. 1995. Regulatory volume decrease in carp red blood cells: mechanisms and oxygenation-dependency of volume-activated potassium and amino acid transport. *J. Exp. Biol.* **198:**155–165
173. Joiner, C.H. 1993. Cation transport and volume regulation in sickle red blood cells. *Am. J Physiol.* **264:**C251–C270
174. Joiner, C.H., Franco, R.S. 2001. The activation of KCl cotransport by deoxygenation and its role in sickle cell dehydration. *Blood Cells Mol. Dis.* **27:**158–164
175. Kaji, D., Kahn, T. 1985. Kinetics of Cl-dependent K influx in human erythrocytes with and without external Na: effect of NEM. *Am. J. Physiol.* **249:**C490–C496
176. Kaji, D.M. 1993. Effect of membrane potential on K-Cl transport in human erythrocytes. *Am. J. Physiol.* **264:**C376–C382
177. Kaji, D.M., Gasson, C. 1995. Urea activation of K-Cl transport in human erythrocytes. *Am. J. Physiol.* **268:**C1018–C1025
178. Kaji, D.M., Tsukitani, Y. 1991. Role of protein phosphatase in activation of KCl cotransport in human erythrocytes. *Am. J. Physiol.* **260:**C176–C180
179. Kakazu, Y., Akaike, N., Komiyama, S., Nabekura, J. 1999. Regulation of intracellular chloride by cotransporters in developing lateral superior olive neurons. *J. Neurosci.* **19:**2843–2851
180. Kakazu, Y., Uchida, S., Nakagawa, T., Akaike, N., Nabekura, J. 2000. Reversibility and cation selectivity of the K^+-Cl^- cotransport in rat central neurons. *J. Neurophysiol.* **84:**281–288
181. Kanagy, N.L., Charpie, J.R., Webb, R.C. 1995. Nitric oxide regulation of ADP-ribosylation of G proteins in hypertension. *Med. Hypotheses.* **44:**159–164
182. Kanaka, C., Ohno, K., Okabe, A., Kuriyama, K., Itoh, T., Fukuda, A., Sato, K. 2001. The differential expression patterns of messenger RNAs encoding K-Cl cotransporters (KCC1, 2) and Na-K-2Cl cotransporter (NKCC1) in the rat nervous system. *Neuroscience.* **104:**933–946
183. Karadsheh, M.F., Byun, N., Mount, D.B., Delpire, E. 2004. Localization of the KCC4 potassium-chloride cotransporter in the nervous system. *Neuroscience.* **123:**381–391
184. Karadsheh, M.F., Delpire, E. 2001. Neuronal restrictive silencing element is found in the KCC2 gene: molecular basis for KCC2-specific expression in neurons. *J. Neurophysiol.* **85:**995–997
185. Kaul, D.K., Fabry, M.E., Nagel, R.L. 1996. The pathophysiology of vascular obstruction in the sickle syndromes. *Blood Rev.* **10:**29–44
186. Kelley, S.J., Dunham, P.B. 1996. Mechanism of swelling activation of K-Cl cotransport in inside-out vesicles of LK sheep erythrocyte membranes. *Am. J. Physiol.* **270:**C1122–C1130
187. Kelley, S.J., Thomas, R., Dunham, P.B. 2000. Candidate inhibitor of the volume-sensitive kinase regulating K-Cl cotransport: the myosin light chain kinase inhibitor ML-7. *J. Membrane Biol.* **178:**31–41
188. Kelsch, W., Hormuzdi, S., Straube, E., Lewen, A., Monyer, H., Misgeld, U. 2001. Insulin-like growth factor 1 and a

cytosolic tyrosine kinase activate chloride outward transport during maturation of hippocampal neurons. *J. Neurosci.* **21:**8339–8347
189. Kemp, P.J., Roberts, G.C., Boyd, C.A. 1994. Identification and properties of pathways for K^+ transport in guinea-pig and rat alveolar epithelial type II cells. *J. Physiol.* **476:**79–88
190. Kennedy, B.C. 1994. Volume regulation in cultured cells derived from human retinal pigment epithelium. *Am. J. Physiol.* **266:**C676–C683
191. Kim, J.A., Kang, Y.S., Lee, Y.S. 2001. Involvement of K^+-Cl^--cotransport in the apoptosis induced by N-ethylmaleimide in HepG2 human hepatoblastoma cells. *Eur. J. Pharmacol.* **418:**1–5
192. Kim, J.A., Kang, Y.Y., Lee, Y.S. 2001. Activation of Na^+, K^+, Cl^- cotransport mediates intracellular Ca^{2+} increase and apoptosis induced by Pinacidil in HepG2 human hepatoblastoma cells. *Biochem. Biophys. Res. Commun.* **281:**511–519
193. Kim, J.A., Lee, Y.S. 2001. Role of reactive oxygen species generated by NADPH oxidase in the mechanism of activation of K^+-Cl^- cotransport by N-ethylmaleimide in HepG2 human hepatoma cells. *Free Radic. Res.* **35:**43–53
194. Kirk, K. 1991. The effect of N-ethylmaleimide on K^+- and Cl^- transport pathways in the lamprey erythrocyte membrane: activation of K^+/Cl^- cotransport. *J. Exp. Biol.* **159:**325–334
195. Kohmoto, O., Krueger, J.A., Barry, W.H. 1990. Activation of furosemide-sensitive K^+ fluxes in myocytes by ouabain and recovery from metabolic inhibition. *Am. J. Physiol.* **259:**H962–H972
196. Kracke, G.R., Dunham, P.B. 1990. Volume-sensitive K-Cl cotransport in inside-out vesicles made from erythrocyte membranes from sheep of low-K phenotype. *Proc. Natl. Acad. Sci. USA.* **87:**8575–8579
197. Kramhoft, B., Lambert, I.H., Hoffmann, E.K., Jorgensen, F. 1986. Activation of Cl-dependent K transport in Ehrlich ascites tumor cells. *Am. J. Physiol.* **251:**C369–C379
198. Krarup, T., Dunham, P.B. 1996. Reconstitution of calyculin-inhibited K-Cl cotransport in dog erythrocyte ghosts by exogenous PP-1. *Am. J. Physiol.* **270:**C898–C902
199. Kregenow, F.M. 1981. Osmoregulatory salt transporting mechanisms: control of cell volume in anisotonic media. *Annu. Rev. Physiol.* **43:**493–505
200. Larsen, F., Solheim, J., Kristensen, T., Kolsto, A.B., Prydz, H. 1993. A tight cluster of five unrelated human genes on chromosome 16q22.1. *Hum. Mol. Genet.* **2:**1589–1595
201. Lauf, P.K. 1982. Evidence for chloride-dependent potassium and water transport induced by hyposmotic stress in erythrocytes of the marine teleost, *Opsanus tau*. *J. Comp. Physiol.* **146:**9–16
202. Lauf, P.K. 1983. Thiol-dependent passive K^+-Cl^- transport in sheep red blood cells. V. Dependence on metabolism. *Am. J. Physiol.* **245:**C445–C448
203. Lauf, P.K. 1984. Thiol-dependent passive K/Cl transport in sheep red cells: IV. Furosemide inhibition as a function of external Rb^+, Na^+, and Cl. *J. Membrane Biol.* **77:**57–62
204. Lauf, P.K. 1985. K^+:Cl^- cotransport: sulfhydryls, divalent cations, and the mechanism of volume activation in a red cell. *J. Membrane Biol.* **88:**1–13
205. Lauf, P.K. 1988. K:Cl cotransport: emerging molecular aspects of a ouabain-resistant, volume-responsive transport system in red blood cells. *Ren. Physiol Biochem.* **11:**248–259
206. Lauf, P.K. 1989. Modulation of K:Cl cotransport by diethylpyrocarbonate. *Prog. Clin. Biol Res.* **292:**339–347
207. Lauf, P.K. 1990. Thiol-dependent passive K: Cl transport in sheep red blood cells: X. A hydroxylamine-oxidation induced K: Cl flux blocked by diethylpyrocarbonate. *J. Membrane Biol.* **118:**153–159
208. Lauf, P.K. 1991. Foreign anions modulate volume set point of sheep erythrocyte K-Cl cotransport. *Am. J. Physiol.* **260:**C503–C512
209. Lauf, P.K. 1992. Incorporation of 3H-N-ethylmaleimide into sheep red cell membrane thiol groups following protection by diamide-induced oxidation. *Mol. Cell Biochem.* **114:**13–20
210. Lauf, P.K. 1998. K^+-Cl^- cotransport: 'to be or not to be' oxygen sensitive. *J. Physiol.* **511:**511
211. Lauf, P.K., Adragna, N.C. 1995. Temperature-induced functional deocclusion of thiols inhibitory for sheep erythrocyte K-Cl cotransport. *Am. J. Physiol.* **269:**C1167–C1175
212. Lauf, P.K., Adragna, N.C. 1996. A thermodynamic study of electroneutral K-Cl cotransport in pH- and volume-clamped low K sheep erythrocytes with normal and low internal magnesium. *J. Gen. Physiol.* **108:**341–350
213. Lauf, P.K., Adragna, N.C. 1998. Functional evidence for a pH sensor of erythrocyte K-Cl cotransport through inhibition by internal protons and diethylpyrocarbonate. *Cell Physiol. Biochem.* **8:**46–60
214. Lauf, P.K., Adragna, N.C. 2000. K-Cl cotransport: properties and molecular mechanism. *Cell Physiol Biochem.* **10:**341–354
215. Lauf, P.K., Adragna, N.C., Agar, N.S. 1995. Glutathione removal reveals kinases as common targets for K-Cl cotransport stimulation in sheep erythrocytes. *Am. J. Physiol.* **269:**C234–C241
216. Lauf, P.K., Adragna, N.C., Garay, R.P. 1984. Activation by N-ethylmaleimide of a latent K^+-Cl^- flux in human red blood cells. *Am. J. Physiol.* **246:**C385–C390
217. Lauf, P.K., Bauer, J., Adragna, N.C., Fujise, H., Zade-Oppen, A.M., Ryu, K.H., Delpire, E. 1992. Erythrocyte K-Cl cotransport: properties and regulation. *Am. J. Physiol.* **263:**C917–C932
218. Lauf, P.K., Erdmann, A., Adragna, N.C. 1994. K-Cl cotransport, pH, and role of Mg in volume-clamped low-K sheep erythrocytes: three equilibrium states. *Am. J. Physiol.* **266:**C95–C103
219. Lauf, P.K., Perkins, C.M., Adragna, N.C. 1985. Cell volume and metabolic dependence of NEM-activated K^+-Cl^- flux in human red blood cells. *Am. J. Physiol.* **249:**C124–C128
220. Lauf, P.K., Theg, B.E. 1980. A chloride dependent K^+ flux induced by N-ethylmaleimide in genetically low-K^+ sheep and goat erythrocytes. *Biochem. Biophys. Res. Commun.* **92:**1422–1428
221. Lauf, P.K., Zhang, J., Delpire, E., Fyffe, R.E., Mount, D.B., Adragna, N.C. 2001. K-Cl co-transport: immunocytochemical and functional evidence for more than one KCC isoform in high K and low K sheep erythrocytes. *Comp. Biochem. Physiol A Mol. Integr. Physiol.* **130:**499–509
222. Lauf, P.K., Zhang, J., Gagnon, K.B., Delpire, E., Fyffe, R.E., Adragna, N.C. 2001. K-Cl cotransport: immunohistochemical and ion flux studies in human embryonic kidney (HEK293) cells transfected with full-length and C- terminal-domain-truncated KCC1 cDNAs. *Cell Physiol Biochem.* **11:**143–160
223. Lawrence, C., Fabry, M.E., Nagel, R.L. 1991. The unique red cell heterogeneity of SC disease: crystal formation, dense reticulocytes, and unusual morphology. *Blood.* **78:**2104–2112
224. Leupen, S.M., Tobet, S.A., Crowley, W.F. Jr., Kaila, K. 2003. Heterogeneous expression of the potassium-chloride cotransporter KCC2 in gonadotropin-releasing hormone neurons of the adult mouse. *Endocrinology.* **144:**3031–3036
225. Levinson, C. 1991. Inability of Ehrlich ascites tumor cells to volume regulate following a hyperosmotic challenge. *J Membrane Biol.* **121:**279–288
226. Lew, V.L., Etzion, Z., Bookchin, R.M. 2002. Dehydration response of sickle cells to sickling-induced Ca^{++} permeabilization. *Blood.* **99:**2578–2585

227. Lew, V.L., Freeman, C.J., Ortiz, O.E., Bookchin, R.M. 1991. A mathematical model of the volume, pH, and ion content regulation in reticulocytes. Application to the pathophysiology of sickle cell dehydration. *J. Clin. Invest.* **87:**100–112
228. Leyssens, A., Dijkstra, S., Van Kerkhove, E., Steels, P. 1994. Mechanisms of K^+ uptake across the basal membrane of malpighian tubules of *Formica polyctena*: the effect of ions and inhibitors. *J. Exp. Biol.* **195:**123–145
229. Li, H., Tornberg, J., Kaila, K., Airaksinen, M.S., Rivera, C. 2002. Patterns of cation-chloride cotransporter expression during embryonic rodent CNS development. *Eur. J. Neurosci.* **16:**2358–2370
230. Liapis, H., Nag, M., Kaji, D.M. 1998. K-Cl cotransporter expression in the human kidney. *Am. J. Physiol.* **275:**C1432–C1437
231. Lincoln, T.M. 1994. Cyclic GMP receptor proteins. *In:* Cyclic GMP: Biochemistry, Physiology and Pathophysiology. MBIU. 5. R. G. Landes Co., Austin, **5:**57–76
232. Lincoln, T.M., Dey, N., Sellak, H. 2001. Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. *J. Appl. Physiol.* **91:**1421–1430
233. Lincoln, T.M., Dey, N.B., Boerth, N.J., Cornwell, T.L., Soff, G.A. 1998. Nitric oxide-cyclic GMP pathway regulates vascular smooth muscle cell phenotypic modulation: implications in vascular diseases. *Acta Physiol. Scand.* **164:**507–515
234. Linton, S.M., O'Donnell, M.J. 1999. Contributions of K^+:Cl^- cotransport and Na^+/K^+-ATPase to basolateral ion transport in malpighian tubules of *Drosophila melanogaster*. *J. Exp. Biol.* **202:**1561–1570
235. Liu, X., Titz, S., Lewen, A., Misgeld, U. 2003. KCC2 mediates NH_4^+ uptake in cultured rat brain neurons. *J. Neurophysiol.* **90:**2785–2790
236. Loeb, J.A., Khurana, T.S., Robbins, J.T., Yee, A.G., Fischbach, G.D. 1999. Expression patterns of transmembrane and released forms of neuregulin during spinal cord and neuromuscular synapse development. *Development.* **126:**781–791
237. Logue, P., Anderson, C., Kanik, C., Farquharson, B., Dunham, P. 1983. Passive potassium transport in LK sheep red cells. Modification by N-ethyl maleimide. *J. Gen. Physiol.* **81:**861–885
238. Lu, J., Karadsheh, M., Delpire, E. 1999. Developmental regulation of the neuronal-specific isoform of K-Cl cotransporter KCC2 in postnatal rat brains. *J. Neurobiol.* **39:**558–568
239. Lytle, C., McManus, T. 2002. Coordinate modulation of Na-K-2Cl cotransport and K-Cl cotransport by cell volume and chloride. *Am. J. Physiol.* **283:**C1422–C1431
240. Macri, P., Breton, S., Beck, J.S., Cardinal, J., Laprade, R. 1993. Basolateral K^+, Cl^-, and HCO_3^- conductances and cell volume regulation in rabbit PCT. *Am. J. Physiol.* **264:**F365–F376
241. Malek, S.A., Coderre, E., Stys, P.K. 2003. Aberrant chloride transport contributes to anoxic/ischemic white matter injury. *J. Neurosci.* **23:**3826–3836
242. Mercado, A., Song, L., Vazquez, N., Mount, D.B., Gamba, G. 2000. Functional comparison of the K^+-Cl^- cotransporters KCC1 and KCC4. *J. Biol Chem.* **275:**30326–30334
243. Merciris, P., Claussen, W.J., Joiner, C.H., Giraud, F. 2003. Regulation of K-Cl cotransport by Syk and Src protein tyrosine kinases in deoxygenated sickle cells. *Pfluegers Arch.* **446:**232–238
244. Merciris, P., Hardy-Dessources, M.D., Sauvage, M., Giraud, F. 1998. Involvement of deoxygenation-induced increase in tyrosine kinase activity in sickle cell dehydration. *Pfluegers Arch.* **436:**315–322
245. Michel, F., Fossat, B., Porthe-Nibelle, J., Lahlou, B., Saint-Marc, P. 1994. Effects of hyposmotic shock on taurine transport in isolated trout hepatocytes. *Exp. Physiol.* **79:**983–995
246. Mikawa, S., Wang, C., Shu, F., Wang, T., Fukuda, A., Sato, K. 2002. Developmental changes in KCC1, KCC2 and NKCC1 mRNAs in the rat cerebellum. *Brain Res. Dev. Brain Res.* **136:**93–100
247. Miyamoto, H., Ikehara, T., Yamaguchi, H., Hosokawa, K., Yonezu, T., Masuya, T. 1986. Kinetic mechanism of Na^+, K^+, Cl^--cotransport as studied by Rb^+ influx into HeLa cells: effects of extracellular monovalent ions. *J. Membrane Biol.* **92:**135–150
248. Morle, L., Pothier, B., Alloisio, N., Feo, C., Garay, R., Bost, M., Delaunay, J. 1989. Reduction of membrane band 7 and activation of volume stimulated (K^+, Cl^--cotransport in a case of congenital stomatocytosis. *Br. J. Haematol.* **71:**141–146
249. Mount, D.B., Delpire, E., Gamba, G., Hall, A.E., Poch, E., Hoover, R.S., Hebert, S.C. 1998. The electroneutral cation-chloride cotransporters. *J. Exp. Biol.* **201:**2091–2102
250. Mount, D.B., Gamba, G. 2001. Renal potassium-chloride cotransporters. *Curr. Opin. Nephrol. Hypertens.* **10:**685–691
251. Mount, D.B., Mercado, A., Song, L., Xu, J., George, A.L. Jr., Delpire, E., Gamba, G. 1999. Cloning and characterization of KCC3 and KCC4, new members of the cation-chloride cotransporter gene family. *J. Biol. Chem.* **274:**16355–16362
252. Movileanu, L., Flonta, M.L., Mihailescu, D., Frangopol, P.T. 1998. Characteristics of ionic transport processes in fish intestinal epithelial cells. *Biosystems.* **45:**123–140
253. Muzyamba, M.C., Gibson, J.S. 2003. Effect of 1-chloro-2,4-dinitrobenzene on K^+ transport in normal and sickle human red blood cells. *J. Physiol.* **547:**903–911
254. Muzyamba, M.C., Speake, P.F., Gibson, J.S. 2000. Oxidants and regulation of K(+)-Cl(−) cotransport in equine red blood cells. *Am. J. Physiol.* **279:**C981–C989
255. Nabekura, J., Ueno, T., Okabe, A., Furuta, A., Iwaki, T., Shimizu-Okabe, C., Fukuda, A., Akaike, N. 2002. Reduction of KCC2 expression and GABAA receptor-mediated excitation after in vivo axonal injury. *J. Neurosci.* **22:**4412–4417
256. Nagel, R.L., Daar, S., Romero, J.R., Suzuka, S.M., Gravell, D., Bouhassira, E., Schwartz, R.S., Fabry, M.E., Krishnamoorthy, R. 1998. HbS-oman heterozygote: a new dominant sickle syndrome. *Blood.* **92:**4375–4382
257. Nagel, R.L., Krishnamoorthy, R., Fattoum, S., Elion, J., Genard, N., Romero, J., Fabry, M.E. 1999. The erythrocyte effects of haemoglobin O(ARAB). *Br. J. Haematol.* **107:**516–521
258. Nagel, R.L., Lawrence, C. 1991. The distinct pathobiology of sickle cell-hemoglobin C disease. Therapeutic implications. *Hematol. Oncol. Clin. North Am.* **5:**433–451
259. Neveu, C., Semblat, J.P., Abad, P., Castagnone-Sereno, P. 2002. Characterization of a cDNA related to K-Cl cotransporters in the root-knot nematode Meloidogyne incognita. *DNA Seq.* **13:**117–121
260. Noblins, M., Kleinknecht, D., Dommergues, J.P., Nazaret, C., Garay, R.P., Jullien, M., Guillot, M., Fries, D., Charpentier, B. 1992. Liddle syndrome (or pseudo-hyperaldosteronism). Long-term development and erythrocyte potassium flow study in 4 cases. *Arch. Fr. Pediatr.* **49:**685–691
261. O'Donnell, M.E. 1989. Regulation of Na-K-Cl cotransport in endothelial cells by atrial natriuretic factor. *Am. J. Physiol.* **257:**C36–C44
262. O'Donnell, M.E. 1991. Endothelial cell sodium-potassium-chloride cotransport. Evidence of regulation by Ca^{2+} and protein kinase C. *J. Biol Chem.* **266:**11559–11566
263. O'Donnell, M.E. 1993. Role of Na-K-Cl cotransport in vascular endothelial cell volume regulation. *Am. J. Physiol.* **264:**C1316–C1326

264. O'Donnell, M.E., Martinez, A., Sun, D. 1995. Cerebral microvascular endothelial cell Na-K-Cl cotransport: regulation by astrocyte-conditioned medium. *Am. J. Physiol.* **268:**C747–C754
265. O'Donnell, M.E., Martinez, A., Sun, D. 1995. Endothelial Na-K-Cl cotransport regulation by tonicity and hormones: phosphorylation of cotransport protein. *Am. J. Physiol.* **269:**C1513–C1523
266. O'Donnell, M.E., Owen, N.E. 1986. Atrial natriuretic factor stimulates Na/K/Cl cotransport in vascular smooth muscle cells. *Proc. Natl. Acad. Sci. USA.* **83:**6132–6136
267. O'Donnell, M.E., Owen, N.E. 1986. Role of cyclic GMP in atrial natriuretic factor stimulation of Na^+, K^+, Cl^- cotransport in vascular smooth muscle cells. *J. Biol. Chem.* **261:**15461–15466
268. Ohnishi, S.T., Ohnishi, T., Ogunmola, G.B. 2001. Green tea extract and aged garlic extract inhibit anion transport and sickle cell dehydration in vitro. *Blood Cells Mol. Dis.* **27:**148–157
269. Olivieri, O., Bonollo, M., Friso, S., Girelli, D., Corrocher, R., Vettore, L. 1993. Activation of K^+ Cl^- cotransport in human erythrocytes exposed to oxidative agents. *Biochim. Biophys. Acta.* **1176:**37–42
270. Olivieri, O., de Franceschi, L., Capellini, M.D., Girelli, D., Corrocher, R., Brugnara, C. 1994. Oxidative damage and erythrocyte membrane transport abnormalities in thalassemias. *Blood.* **84:**315–320
271. Olivieri, O., de Franceschi, L., de Gironcoli, M., Girelli, D., Corrocher, R. 1993. Potassium loss and cellular dehydration of stored erythrocytes following incubation in autologous plasma: role of the KCl cotransport system. *Vox Sang.* **65:**95–102
272. Olivieri, O., Vitoux, D., Bachir, D., Beuzard, Y. 1991. K^+ efflux in deoxygenated sickle cells in the presence or absence of DIOA, a specific inhibitor of the [K^+, Cl^-] cotransport system. *Br. J. Haematol.* **77:**117–120
273. Olivieri, O., Vitoux, D., Galacteros, F., Bachir, D., Blouquit, Y., Beuzard, Y., Brugnara, C. 1992. Hemoglobin variants and activity of the (K^+ Cl^-) cotransport system in human erythrocytes. *Blood.* **79:**793–797
274. Olson, J.E., Alexander, C., Feller, D.A., Clayman, M.L., Ramnath, E.M. 1995. Hypoosmotic volume regulation of astrocytes in elevated extracellular potassium. *J. Neurosci. Res.* **40:**333–342
275. O'Neill, W.C. 1989. Cl-dependent K transport in a pure population of volume-regulating human erythrocytes. *Am. J. Physiol.* **256:**C858–C864
276. O'Neill, W.C. 1991. Swelling-activated K-Cl cotransport: metabolic dependence and inhibition by vanadate and fluoride. *Am. J. Physiol.* **260:**C308–C315
277. O'Reilly, N., Xia, Z., Fiander, H., Tauskela, J., Small, D.L. 2002. Disparity between ionic mediators of volume regulation and apoptosis in N1E 115 mouse neuroblastoma cells. *Brain Res.* **943:**245–256
278. Orlov, S.N., Kolosova, I.A., Cragoe, E.J., Gurlo, T.G., Mongin, A.A., Aksentsev, S.L., Konev, S.V. 1993. Kinetics and peculiarities of thermal inactivation of volume-induced Na^+/H^+ exchange, Na^+, K^+, $2Cl^-$ cotransport and K^+,Cl^- cotransport in rat erythrocytes. *Biochim. Biophys. Acta.* **1151:**186–192
279. Orlov, S.N., Kuznetsov, S.R., Kolosova, I.A., Aksentsev, S.L., Konev, S.V. 1997. Volume-dependent regulation of ion carriers in human and rat erythrocytes: Role of cytoskeleton and protein phosphorylation. *Russian J. Physiol.* **83:**134–147
280. Orlov, S.N., Tremblay, J., Hamet, P. 1996. Bumetanide-sensitive ion fluxes in vascular smooth muscle cells: lack of functional Na^+, K^+, $2Cl^-$ cotransport. *J. Membrane Biol.* **153:**125–135
281. Orringer, E.P. 1984. A further characterization of the selective K movements observed in human red blood cells following acetylphenylhydrazine exposure. *Am. J. Hematol.* **16:**355–366
282. Orringer, E.P., Brockenbrough, J.S., Whitney, J.A., Glosson, P.S., Parker, J.C. 1991. Okadaic acid inhibits activation of K-Cl cotransport in red blood cells containing hemoglobins S and C. *Am. J. Physiol.* **261:**C591–C593
283. Ortiz-Carranza, O., Adragna, N.C., Lauf, P.K. 1996. Modulation of K-Cl cotransport in volume-clamped low-K sheep erythrocytes by pH, magnesium, and ATP. *Am. J. Physiol.* **271:**C1049–C1058
284. Parker, J.C. 1992. Volume-activated cation transport in dog red cells: detection and transduction of the volume stimulus. *Comp. Biochem. Physiol. Comp. Physiol.* **102:**615–618
285. Parker, J.C. 1993. Urea alters set point volume for K-Cl cotransport, Na-H exchange, and Ca-Na exchange in dog red blood cells. *Am. J. Physiol.* **265:**C447–C452
286. Parker, J.C., Colclasure, G.C. 1992. Actions of thiocyanate and N-phenylmaleimide on volume-responsive Na and K transport in dog red cells. *Am. J. Physiol.* **262:**C418–C421
287. Parker, J.C., Colclasure, G.C. 1992. Macromolecular crowding and volume perception in dog red cells. *Mot. Cell Biochem.* **114:**9–11
288. Parker, J.C., Colclasure, G.C., McManus, T.J. 1991. Coordinated regulation of shrinkage-induced Na/H exchange and swelling-induced [K-Cl] cotransport in dog red cells. Further evidence from activation kinetics and phosphatase inhibition. *J. Gen. Physiol.* **98:**869–880
289. Parker, J.C., Dunham, P.B., Minton, A.P. 1995. Effects of ionic strength on the regulation of Na/H exchange and K-Cl cotransport in dog red blood cells. *J. Gen. Physiol.* **105:**677–699
290. Parker, J.C., McManus, T.J., Starke, L.C., Gitelman, H.J. 1990. Coordinated regulation of Na/H exchange and [K-Cl] cotransport in dog red cells. *J. Gen. Physiol.* **96:**1141–1152
291. Pasantes-Morales, H., Moran, J., Schousboe, A. 1990. Volume-sensitive release of taurine from cultured astrocytes: properties and mechanism. *Glia.* **3:**427–432
292. Payne, J.A. 1997. Functional characterization of the neuronal-specific K-Cl cotransporter: implications for $[K^+]_o$ regulation. *Am. J. Physiol.* **273:**C1516–C1525
293. Payne, J.A., Stevenson, T.J., Donaldson, L.F. 1996. Molecular characterization of a putative K-Cl cotransporter in rat brain. A neuronal-specific isoform. *J. Biol. Chem.* **271:**16245–16252
294. Pearson, M.M., Lu, J., Mount, D.B., Delpire, E. 2001. Localization of the K^+-Cl^- cotransporter, KCC3, in the central and peripheral nervous systems: expression in the choroid plexus, large neurons and white matter tracts. *Neuroscience.* **103:**481–491
295. Pellegrino, C.M., Rybicki, A.C., Musto, S., Nagel, R.L., Schwartz, R.S. 1998. Molecular identification and expression of erythroid K:Cl cotransporter in human and mouse erythroleukemic cells. *Blood Cells Mol. Dis.* **24:**31–40
296. Perry, P.B., O'Neill, W.C. 1993. Swelling-activated K fluxes in vascular endothelial cells: volume regulation via K-Cl cotransport and K channels. *Am. J. Physiol.* **265:**C763–C769
297. Peters, L.L., Jindel, H.K., Gwynn, B., Korsgren, C., John, K.M., Lux, S.E., Mohandas, N., Cohen, C.M., Cho, M.R., Golan, D.E., Brugnara, C. 1999. Mild spherocytosis and altered red cell ion transport in protein 4. 2-null mice. *J. Clin. Invest.* **103:**1527–1537
298. Piechotta, K., Lu, J., Delpire, E. 2002. Cation chloride cotransporters interact with the stress-related kinases Ste20-related proline-alanine-rich kinase (SPAK) and oxidative stress response 1 (OSR1). *J. Biol. Chem.* **277:**50812–50819

299. Piwnica-Worms, D., Jacob, R., Horres, C.R., Lieberman, M. 1985. Potassium-chloride cotransport in cultured chick heart cells. *Am. J. Physiol.* **249:**C337–C344
300. Poillon, W.N., Kim, B.C., Labotka, R.J., Hicks, C.U., Kark, J.A. 1995. Antisickling effects of 2,3-diphosphoglycerate depletion. *Blood.* **85:**3289–3296
301. Race, J.E., Makhlouf, F.N., Logue, P.J., Wilson, F.H., Dunham, P.B., Holtzman, E.J. 1999. Molecular cloning and functional characterization of KCC3, a new K-Cl cotransporter. *Am. J. Physiol.* **277:**C1210–C1219
302. Reid, K.H., Li, G.Y., Payne, R.S., Schurr, A., Cooper, N.G. 2001. The mRNA level of the potassium-chloride cotransporter KCC2 covaries with seizure susceptibility in inferior colliculus of the post-ischemic audiogenic seizure-prone rat. *Neurosci. Lett.* **308:**29–32
303. Rivera, C., Li, H., Thomas-Crusells, J., Lahtinen, H., Viitanen, T., Nanobashvili, A., Kokaia, Z., Airaksinen, M.S., Voipio, J., Kaila, K., Saarma, M. 2002. BDNF-induced TrkB activation down-regulates the K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl- extrusion. *J. Cell Biol.* **159:**747–752
304. Rivera, C., Voipio, J., Payne, J.A., Ruusuvuori, E., Lahtinen, H., Lamsa, K., Pirvola, U., Saarma, M., Kaila, K. 1999. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature.* **397:**251–255
305. Romero, J.R., Fabry, M.E., Suzuka, S.M., Costantini, F., Nagel, R.L., Canessa, M. 1997. K:Cl cotransport in red cells of transgenic mice expressing high levels of human hemoglobin S. *Am. J. Hematol.* **55:**112–114
306. Romero, J.R., Suzuka, S.M., Nagel, R.L., Fabry, M.E. 2003. Expression of HbC and HbS, but not Hb A, results in activation of K-Cl cotransport activity in transgenic mouse red cells. *Blood.* **103:**2384–2390
307. Romero, J.R., Suzuka, S.M., Romero-Gonzalez, G.V., Nagel, R.L., Fabry, M.E. 2001. K:Cl cotransport activity is inhibited by HCO_3^- in knockout mouse red cells expressing human HbC. *Blood Cells Mol. Dis.* **27:**69–70
308. Rota, R., Nazaret, C., Henrotte, J.G., Garay, R.P., Guicheney, P. 1991. Dissociation between derepressed K^+, Cl^- cotransport system and high blood pressure in the F2 hybrid generation (SHR × WKY). *J. Hypertens. Suppl.* **9:**S298–S299
309. Russel, J.M. 2000. Sodium-potassium-chloride cotransport. *Physiol. Revs.* **80:**211–276
310. Ryu, K.H., Lauf, P.K. 1990. Evidence for inhibitory SH groups in the thiol activated K:Cl cotransporter of low K sheep red blood cells. *Mol. Cell Biochem.* **99:**135–140
311. Sachs, J.R. 1994. Soluble polycations and cationic amphiphiles inhibit volume-sensitive K-Cl cotransport in human red cell ghosts. *Am. J. Physiol.* **266:**C997–C1005
312. Sachs, J.R., Martin, D.W. 1993. The role of ATP in swelling-stimulated K-Cl cotransport in human red cell ghosts. Phosphorylation-dephosphorylation events are not in the signal transduction pathway. *J. Gen. Physiol.* **102:**551–573
313. Sachs, J.R., Martin, D.W. 1999. Role of polyamine structure in inhibition of K^+-Cl^- cotransport in human red cell ghosts. *J. Physiol.* **520:**723–735
314. Saitta, M., Cavalier, S., Garay, R., Cragoe, E. Jr., Hannaert, P. 1990. Evidence for a DIOA-sensitive [K^+, Cl]-cotransport system in cultured vascular smooth muscle cells. *Am. J. Hypertens.* **3:**939–942
315. Saitta, M.N., Hannaert, P.A., Rosati, C., Diaz, A.S., Senn, N., Garay, R.P. 1988. [Na^+, K^+, Cl^-]-cotransport function and dysfunction in different forms of primary hypertension. *Am. J. Hypertens.* **1:**60S–63S
316. Sallinen, R., Tornberg, J., Putkiranta, M., Horelli-Kuitunen, N., Airaksinen, M.S., Wessman, M. 2001. Chromosomal localization of SLC12A5/Slcl2a5, the human and mouse genes for the neuron-specific K^+-Cl^- cotransporter (KCC2) defines a new region of conserved homology. *Cytogenet. Cell Genet.* **94:**67–70
317. Sandstrom, P.E., Sehlin, J. 1993. Evidence for separate Na^+, K^+, Cl and K^+, Cl co-transport systems in mouse pancreatic beta-cells. *Eur. J. Pharmacol.* **238:**403–405
318. Sangan, P., Brill, S.R., Sangan, S., Forbush, B. 3rd., Binder, H.J. 2000. Basolateral K-Cl cotransporter regulates colonic potassium absorption in potassium depletion. *J. Biol. Chem.* **275:**30813–30816
319. Santoso, A.W., Scott, D.M., Kinne, R. 1987. Localization of Tamm-Horsfall protein in chloride transporting epithelia: lack of correlation with the Na, K, Cl cotransporter. *Eur. J. Cell Biol.* **43:**104–109
320. Sarkadi, B., Parker, J.C. 1991. Activation of ion transport pathways by changes in cell volume. *Biochim. Biophys. Acta.* **1071:**407–427
321. Sarkar, R., Meinberg, E.G., Stanley, J.C., Gordon, D., Webb, R.C. 1996. Nitric oxide reversibly inhibits the migration of vascular smooth muscle cells. *Circ. Res.* **78:**225–230
322. Schoenherr, C.J., Paquette, A.J., Anderson, D.J. 1996. Identification of potential target genes for neuron-restrictive silencer factor. *Proc. Natl. Acad. Sci. USA.* **93:**9881–9886
323. Schultz, J., Milpetz, F., Bork, P., Ponting, C.P. 1998. SMART, a simple modular architecture research tool: Identification of signaling domains. *Proc. Natl. Acad. Sci. USA.* **95:**5857–5864
324. Schwartz, R.S., Musto, S., Fabry, M.E., Nagel, R.L. 1998. Two distinct pathways mediate the formation of intermediate density cells and hyperdense cells from normal density sickle red blood cells. *Blood.* **92:**4844–4855
325. Seki, G., Taniguchi, S., Uwatoko, S., Suzuki, K., Kurokawa, K. 1993. Evidence for conductive Cl^- pathway in the basolateral membrane of rabbit renal proximal tubule S3 segment. *J. Clin. Invest.* **92:**1229–1235
326. Shen, M.R., Chou, C.Y., Ellory, J.C. 2000. Volume-sensitive KCl cotransport associated with human cervical carcinogenesis. *Pfluegers Arch.* **440:**751–760
327. Shen, M.R., Chou, C.Y., Hsu, K.F., Hsu, Y.M., Chiu, W.T., Tang, M.J., Alper, S.L., Ellory, J.C. 2003. KCl cotransport is an important modulator of human cervical cancer growth and invasion. *J. Biol. Chem.* **278:**39941–39950
328. Shen, M.R., Chou, C.Y., Hsu, K.F., Liu, H.S., Dunham, P.B., Holtzman, E.J., Ellory, J.C. 2001. The KCl cotransporter isoform KCC3 can play an important role in cell growth regulation. *Proc. Natl. Acad. Sci. USA.* **98:**14714–14719
329. Shennan, D.B., McNeillie, S.A. 1990. Efflux of chloride from lactating rat mammary tissue slices. *Comp. Biochem. Physiol. A.* **95:**367–371
330. Shmukler, B.E., Brugnara, C., Alper, S.L. 2000. Structure and genetic polymorphism of the mouse KCC1 gene. *Biochim. Biophys. Acta.* **1492:**353–361
331. Simmons, N.L., Tivey, D.R. 1992. The effect of hyperosmotic challenge upon ion transport in cultured renal epithelial layers (MDCK). *Pfluegers Arch.* **421:**503–509
332. Simons, K., Toomre, D. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1:**31–39
333. Song, L., Mercado, A., Vazquez, N., Xie, Q., Desai, R., George, A.L. Jr., Gamba, G., Mount, D.B. 2002. Molecular, functional, and genomic characterization of human KCC2, the neuronal K-Cl cotransporter. *Brain Res. Mol. Brain Res.* **103:**91–105

334. Soszynski, M., Bartosz, G. 1997. Effect of postirradiation treatment on the radiation-induced haemolysis of human erythrocytes. *Int. J. Radiat. Biol.* **71:**337–343
335. Speake, P.F., Gibson, J.S. 1997. Urea-stimulated K-Cl cotransport in equine red blood cells. *Pfluegers Arch.* **434:**104–112
336. Speake, P.F., Roberts, C.A., Gibson, J.S. 1997. Effect of changes in respiratory blood parameters on equine red blood cell K-Cl cotransporter. *Am. J. Physiol.* **273:**C1811–C1818
337. Starke, L.C., Jennings, M.L. 1993. K-Cl cotransport in rabbit red cells: further evidence for regulation by protein phosphatase type 1. *Am. J. Physiol.* **264:**C118–C1124
338. Steinlein, O.K., Neubauer, B.A., Sander, T., Song, L., Stoodt, J., Mount, D.B. 2001. Mutation analysis of the potassium chloride cotransporter KCC3 (SLC12A6) in rolandic and idiopathic generalized epilepsy. *Epilepsy Res.* **44:**191–195
339. Strange, K., Singer, T.D., Morrison, R., Delpire, E. 2000. Dependence of KCC2 K-Cl cotransporter activity on a conserved carboxy terminus tyrosine residue. *Am. J. Physiol.* **279:**C860–C867
340. Su, W., Shmukler, B.E., Chernova, M.N., Stuart-Tilley, A.K., de Franceschi, L., Brugnara, C., Alper, S.L. 1999. Mouse K-Cl cotransporter KCC1: cloning, mapping, pathological expression, and functional regulation. *Am. J. Physiol.* **277:**C899–C912
341. Sugihara, T., Hebbel, R.P. 1992. Exaggerated cation leak from oxygenated sickle red blood cells during deformation: evidence for a unique leak pathway. *Blood.* **80:**2374–2378
342. Takeuchi, S., Ando, M., Irimajiri, A. 1997. Changes in the volume of marginal cells induced by isotonic 'Cl depletion/restoration': involvement of the Cl channel and Na^+-K^+-Cl^- cotransporter. *Hear. Res.* **113:**99–109
343. Taouil, K., Hannaert, P. 1999. Evidence for the involvement of K^+ channels and K^+-Cl^- cotransport in the regulatory volume decrease of newborn rat cardiomyocytes. *Pfluegers Arch.* **439:**56–66
344. Theander, S., Edman, A., Fahraeus, C., Akoev, G.N., Grampp, W. 1999. Cl^- transport in the lobster stretch receptor neurone. *Acta Physiol. Scand.* **167:**285–298
345. Thomas, S., Egee, S. 1998. Fish red blood cells: characteristics and physiological role of the membrane ion transporters. *Comp. Biochem. Physiol A Mol. Integr. Physiol.* **119:**79–86
346. Tosco, M., Orsenigo, M.N., Faelli, A. 1995. Bicarbonate and chloride transport across rat ileal basolateral membrane. *Experientia.* **51:**799–803
347. Ueno, T., Okabe, A., Akaike, N., Fukuda, A., Nabekura, J. 2002. Diversity of neuron-specific K^+-Cl^- cotransporter expression and inhibitory postsynaptic potential depression in rat motoneurons. *J. Biol Chem.* **277:**4945–4950
348. Vardi, N., Zhang, L.L., Payne, J.A., Sterling, P. 2000. Evidence that different cation chloride cotransporters in retinal neurons allow opposite responses to GABA. *J. Neurosci.* **20:**7657–7663
349. Velazquez, H., Ellison, D.H., Wright, F.S. 1992. Luminal influences on potassium secretion: chloride, sodium, and thiazide diuretics. *Am. J. Physiol.* **262:**F1076–F1082
350. Velazquez, H., Silva, T. 2003. Cloning and localization of KCC4 in rabbit kidney: expression in distal convoluted tubule. *Am. J. Physiol.* **285:**F49–F58
351. Vitoux, D., Beuzard, Y., Brugnara, C. 1999. The effect of hemoglobin A and S on the volume- and pH-dependence of K-Cl cotransport in human erythrocyte ghosts. *J. Membrane Biol.* **167:**233–240
352. Vitoux, D., Olivieri, O., Garay, R.P., Cragoe, E.J. Jr., Galacteros, F., Beuzard, Y. 1989. Inhibition of K^+ efflux and dehydration of sickle cells by [(dihydroindenyl)oxy]alkanoic acid: an inhibitor of the K^+Cl^- cotransport system. *Proc. Natl. Acad. Sci. USA.* **86:**4273–4276
353. Von Brauchitsch, D.K., Crook, R.B. 1993. Protein kinase C regulation of a Na^+, K^+, Cl^- cotransporter in fetal human pigmented ciliary epithelial cells. *Exp. Eye Res.* **57:**699–708
354. Vu, T.Q., Payne, J.A., Copenhagen, D.R. 2000. Localization and developmental expression patterns of the neuronal K-Cl cotransporter (KCC2) in the rat retina. *J. Neurosci.* **20:**1414–1423
355. Warnock, D.G., Eveloff, J. 1989. K-Cl cotransport systems. *Kidney Int.* **36:**412–417
356. Weil-Maslansky, E., Gutman, Y., Sasson, S. 1994. Insulin activates furosemide-sensitive K^+ and Cl^- uptake system in BC3H1 cells. *Am. J. Physiol.* **267:**C932–C939
357. Weinstein, A.M. 1983. Nonequilibrium thermodynamic model of the rat proximal tubule epithelium. *Biophys. J.* **44:**153–170
358. Weinstein, A.M. 1992. Chloride transport in a mathematical model of the rat proximal tubule. *Am. J. Physiol.* **263:**F784–F798
359. White, R.E., Darkow, D.J., Lang, J.L. 1995. Estrogen relaxes coronary arteries by opening BK_{Ca} channels through a cGMP-dependent mechanism. *Circ. Res.* **77:**936–942
360. Wiater, L.A., Dunham, P.B. 1983. Passive transport of K^+ and Na^+ in human red blood cells: sulfhydryl binding agents and furosemide. *Am. J. Physiol.* **245:**C348–C356
361. Williams, J.R., Sharp, J.W., Kumari, V.G., Wilson, M., Payne, J.A. 1999. The neuron-specific K-Cl cotransporter, KCC2. Antibody development and initial characterization of the protein. *J. Biol. Chem.* **274:**12656–12664
362. Willis, J.S., Anderson, G.L. 1998. Activation of K-Cl cotransport by mild warming in guinea pig red cells. *J. Membrane Biol.* **163:**193–203
363. Wolpaw, E.W., Martin, D.L. 1984. Cl- transport in a glioma cell line: evidence for two transport mechanisms. *Brain Res.* **297:**317–327
364. Wolpaw, E.W., Martin, D.L. 1986. Sulfate-chloride exchange transport in a glioma cell line. *Biochim. Biophys. Acta.* **855:**302–311
365. Woo, N.S., Lu, J., England, R., McClellan, R., Dufour, S., Mount, D.B., Deutch, A.Y., Lovinger, D.M., Delpire, E. 2002. Hyperexcitability and epilepsy associated with disruption of the mouse neuronal-specific K-Cl cotransporter gene. *Hippocampus.* **12:**258–268
366. Yaffe, M.B., Leparc, G.G., Lai, J., Obata, T., Volinia, S., Cantley, L.C. 2001. A motif-based profile scanning approach for genome-wide prediction of signaling pathways. *Nature Biotechnol.* **19:**348–353
367. Yamada, Y., Fukuda, A., Tanaka, M., Shimano, Y., Nishino, H., Muramatsu, K., Togari, H., Wada, Y. 2001. Optical imaging reveals cation-Cl^- cotransporter-mediated transient rapid decrease in intracellular Cl^- concentration induced by oxygen-glucose deprivation in rat neocortical slices. *Neurosci. Res.* **39:**269–280
368. Yan, G.X., Chen, J., Yamada, K.A., Kleber, A.G., Corr, P.B. 1996. Contribution of shrinkage of extracellular space to extracellular K^+ accumulation in myocardial ischaemia of the rabbit. *J. Physiol.* **490:**215–228
369. Zade-Oppen, A.M., Lauf, P.K. 1990. Thiol-dependent passive K:Cl transport in sheep red blood cells: IX. Modulation by pH in the presence and absence of DIDS and the effect of NEM. *J. Membrane Biol.* **118:**143–151

370. Zeuthen, T. 1994. Cotransport of K^+ Cl^- and H_2O by membrane proteins from choroid plexus epithelium of *Necturus maculosus*. *J. Physiol.* **478:**203–219
371. Zeuthen, T. 2000. Molecular water pumps. *Rev. Physiol. Biochem. Pharmacol.* **141:**97–151
372. Zhang, J., Lauf, P.K., Adragna, N.C. 2001. Serum affects Rb influx through Cl-dependent and Cl-independent pathways in primary cultured vascular smooth muscle cells (VSMCs). *FASEB J.* **15:**A114
373. Zhang, J., Lauf, P.K., Adragna, N.C. 2003. Platelet-derived growth factor regulates K-Cl cotransport in vascular smooth muscle cells. *Am. J. Physiol.* **284:**C674–C680
374. Zhang, J., Lauf, P.K., Adragna, N.C. 2003. Post-translational regulation of K-Cl cotransport (COT) by platelet-derived growth factor (PDGF) is mediated by the phosphoinositide 3-kinase (PI3-K) in vascular smooth muscle cells (VSMCs). *FASEB J.* **17:**A49
375. Zhou, Z.J. 2001. A critical role of the strychnine-sensitive glycinergic system in spontaneous retinal waves of the developing rabbit. *J. Neurosci.* **21:**5158–5168