NKCC1 and NKCC2: The pathogenetic role of cation-chloride cotransporters in hypertension

Sergei N. Orlov\textsuperscript{a,b,*}, Svetlana V. Koltsova\textsuperscript{a}, Leonid V. Kapilevich\textsuperscript{b,c}, Svetlana V. Gusakova\textsuperscript{c}, and Nickolai O. Dulin\textsuperscript{d}

\textsuperscript{a}Faculty of Biology, M.V. Lomonosov Moscow State University, Russia
\textsuperscript{b}Tomsk State University, Russia
\textsuperscript{c}Siberian State Medical University, Russia
\textsuperscript{d}University of Chicago Department of Medicine, USA

Abstract

This review summarizes the data on the functional significance of ubiquitous (NKCC1) and renal-specific (NKCC2) isoforms of electroneutral sodium, potassium and chloride cotransporters. These carriers contribute to the pathogenesis of hypertension via regulation of intracellular chloride concentration in vascular smooth muscle and neuronal cells and via sensing chloride concentration in the renal tubular fluid, respectively. Both NKCC1 and NKCC2 are inhibited by furosemide and other high-ceiling diuretics widely used for attenuation of extracellular fluid volume. However, the chronic usage of these compounds for the treatment of hypertension and other volume-expanded disorders may have diverse side-effects due to suppression of myogenic response in microcirculatory beds.

Keywords

hypertension; intracellular chloride; myogenic tone; neuronal cell; NKCC cotransport; smooth muscle

\textsuperscript{Na}\textsuperscript{+}, \textsuperscript{K}\textsuperscript{+}, 2\textsuperscript{Cl}\textsuperscript{−} cotransporters (NKCC) encoding by \textit{SLC12A2} (NKCC1) and \textit{SLC12A1} (NKCC2) belong to the subfamily of cation–chloride cotransporters (CCCs) that provide electroneutral transport of sodium, potassium and chloride across the plasma membrane and are inhibited by bumetanide, furosemide and several other structurally similar compounds (Table 1). Because the major target of these drugs is inhibition of ion transport in thick ascending limb of Henle’s loop (TAHL), they were termed as high-ceiling diuretics. Gene structure, membrane architecture and pharmacology of CCCs were subjected to detailed

Copyright © 2015, Chongqing Medical University. Production and hosting by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
\textsuperscript{*}Corresponding author. Laboratory of Biomembranes, Room 169, Faculty of Biology, MSU, Vorob’evy Gory 1, Build 12, Moscow 199899, Russia., sergeinorlov@yandex.ru (S.N. Orlov).
Peer review under responsibility of Chongqing Medical University.
Conflicts of interest
All authors have none to declare.
analysis in several comprehensive reviews. This review focuses on physiological significance of NKCCs and their involvement in the pathogenesis of hypertension via regulation of intracellular chloride concentration and sensing chloride concentration in the renal tubular fluid.

**Functional implications of NKCCs**

**Cell volume regulation**

Animal cells maintain their volume with an accuracy of 1%–2% by means of systems providing inwardly and outwardly directed fluxes of monovalent ions and organic osmolytes and termed as regulatory volume increase (RVI) and regulatory volume decrease (RVD), respectively. In early studies with mammalian erythrocytes, we and others have shown that cell shrinkage and swelling result in activation of NKCC and K⁺, Cl⁻ cotransport (KCC), respectively. We also demonstrated that in vascular smooth muscle cells (VSMC) subjected to hyperosmotic shrinkage, RVI is caused by activation of NKCC. It is noteworthy that under the baseline isosmotic conditions, inhibition of NKCC by bumetanide does not significantly affect the volume of human lung fibroblasts (Table 2), suggesting that in the absence of external stimuli, NKCC has a minor impact on the generation of net osmolite fluxes in these cells. However, under inhibition of the Na⁺, K⁺-ATPase by ouabain, treatment with bumetanide resulted in ~2-fold elevation in the volume of human lung fibroblasts (Table 2). This observation suggests that dissipation of transmembrane gradient of monovalent cations evoked by Na⁺, K⁺-ATPase inhibition results in generation of inwardly-directed ion flux mediated by NKCC1, the only isoform of NKCC expressed in these cells. Functional consequences of the disturbances of cell volume regulatory machinery are discussed elsewhere.

**NKCC as a regulator of intracellular Cl⁻ concentration**

In all types of cells studied so far, CCCs generate both inwardly and outwardly-directed ion movements and the direction of net flux depends on carrier’s stoichiometry and transmembrane gradients of cations created by Na⁺, K⁺-ATPase. Thus, the stoichiometry 1:1 predicts that the value of ion flux is in direct proportion to concentration of co-transporting ions. Because [Na⁺]o ≫ [Na⁺]i, [K⁺]i ≫ [K⁺]o and [Cl⁻]o > [Cl⁻]i, net fluxes generated by Na⁺, Cl⁻ cotransport (NCC) and KCC exhibit inward and outward directions, respectively. In the majority of cells [Cl⁻]o > [Cl⁻]i and net flux mediated by electroneutral NKCC functioning with the stoichiometry 1Na⁺:1K⁺:2Cl⁻ has inward direction.

The above consideration suggests that CCCs play a key role in the regulation of [Cl⁻]i whereas their contribution to the adjustment of intracellular concentration of monovalent cations is negligible because of the highly active Na⁺, K⁺-pump. Indeed, it was shown that inhibition of NCC and NKCC results in attenuation of [Cl⁻]i whereas suppression of KCC activity increases this parameter. Importantly, the alteration of CCC activity may lead to adjustment of [Cl⁻]i above or below the values corresponding to Nernst’s equilibrium potential. This means that in cells abundant with anion channels, CCCs contribute to maintenance of electrical potential thus having an impact on the whole spectrum of cellular

*Genes Dis. Author manuscript; available in PMC 2015 June 23.*
functions controlled by potential-sensitive proteins localized within the plasma membrane. This conclusion is supported by data discussed in the next sections.

**VSMC contraction**

In skeletal and cardiac muscle, plasma membrane permeability for $K^+$ ($P_K$) plays a major role in the maintenance of electrical resistance and resting potential ($E_m$). In contrast, the values of $P_K$ and $P_{Cl}$ in smooth muscles are about the same, suggesting that in VSMC CCCs may be involved in regulation of the $[Cl^-]/[Cl^-]_o$ ratio and therefore of $E_m$ and excitation-contraction coupling. Indeed, NKCC inhibition by furosemide or bumetanide resulted in a decreased $[Cl^-]$, and led to hyperpolarization of rat VSMC. These data suggest that decreased baseline tone seen in smooth muscles treated with Henle’s loop diuretics, as well as attenuation by these compounds of contraction of smooth muscle strips evoked by modest increment of $[K^+]_o$, by electrical stimulation, by histamine, angiotensin II, thromboxane A2, oxitocyn, α-adrenergic, or purinergic agonists is caused by $Cl^-$-dependent hyperpolarization and suppression of the activity of voltage-gated L-type Ca$^{2+}$ channels.

**Myogenic response**

Myogenic tone (response) is a unique property of small (<100–200 μm) blood vessels to decrease rather than increase their inner diameter in response to elevated intra-luminal pressure. Both kinetic and the amplitude of myogenic response are different in blood vessels of different origins. Importantly, myogenic response in blood vessels from the brain, skeletal muscle and renal afferent arteriole provides constant blood supply of these tissues independently of the changes in the systemic blood pressure.

It was reported that bumetanide decreased the myogenic tone of mesenteric arteries and completely abolished myogenic response of renal afferent arteriole. We demonstrated that inhibitory action of bumetanide (but not of L-type Ca$^{2+}$ channel blocker nicardipine) on the myogenic response as well as on contraction triggered by α-adrenergic stimulation is absent in mesenteric arteries from NKCC1$^{-/-}$ mice. Because NKCC2 is not expressed in SMC, these data suggest that bumetanide and other loop’s diuretics inhibit contraction and myogenic response of vascular SMC via their interaction with NKCC1, i.e., an ubiquitous isoform Na$^+$, $K^+$, 2Cl$^-\,$ cotransporter.

**Synaptic transmission**

Neuron–neuron interactions are controlled by neurotransmitters via regulation of transduction of electrical signals. Excitatory and inhibitory neurotransmitters lead to depolarization and hyperpolarization of postsynaptic membrane, respectively. For example, ionotropic glutamate receptor and acetylcholine receptors cause depolarization of postsynaptic membrane via increment of ion current mediated by ion channels permeable for Na$^+$ and Ca$^{2+}$. In contrast, hyperpolarization results from the increment of the permeability of K$^+$ channels triggered by activation of metabotropic acetylcholine receptors. Unlike above-listed neurotransmitters, gamma-aminobutiric acid (GABA) increases permeability for Cl$^-$ and other low molecular weight anions via its interaction with ionotropic GABA$\ _A$ receptors. Direction of net flux mediated by these receptors is determined by transmembrane
chloride gradient and electrical potential of postsynaptic membrane. In case RT/F × ln([Cl\textsuperscript{−}\textsubscript{o}]/[Cl\textsuperscript{−}\textsubscript{i}] < E\textsubscript{m}, the net chloride flux will be directed into the cells which will lead to hyperpolarization and attenuation of neuronal activity. An elevation of [Cl\textsuperscript{−}\textsubscript{i}] changes the direction of net chloride flux and in this case activation of GABA\textsubscript{A} receptors leads to elevation of neuronal activity.

Keeping this in mind, one may assume that the ratio of activity of NKCC1 and KCC2, providing inwardly and outwardly directed Cl\textsuperscript{−} fluxes in neuronal cells, respectively, plays a key role in the function of GABA\textsubscript{A} receptors. Indeed, the attenuation of NKCC1 activity on the background of elevated KCC2 is the major mechanism of the alteration of the functional properties of GABA\textsubscript{A} receptors in central neuronal system (CNS) of mammals during ontogenesis: GABA\textsubscript{A} receptors function as activatory receptors in prenatal stage but became inhibitory ones few days after birth (for review see\textsuperscript{34–36}).

**Salt reabsorption by NKCC2**

A major impact of NKCC in salt reabsorption and regulation of extracellular fluid volume (EFV) is well-established by the clinical use of furosemide and other inhibitors of these carriers as potent diuretics,\textsuperscript{37} as well as by salt-lasting characteristic of the loss-off-function mutations of NKCC2 that underpin type I Barter syndrome.\textsuperscript{38} Importantly, in addition to salt reabsorption in the TAHL, NKCC2 contributes to the regulation of salt excretion via tubuloglomerular feedback (TGF) in the juxtaglomerular apparatus (JGA) neighbouring to the distal segment of TAHL and consisting of epithelial cells of macula densa (MD), mesangial cells and VSMC. Such location is unique along the nephron and plays a key role in the JGA function. Indeed, [NaCl] in renal fluid delivered to the MD is in the range of 20–60 mM. This is in sharp contrast to the proximal tubule, where concentrations of most solutes deviate modestly from those in plasma (for more details, see\textsuperscript{39–41}). TGF is triggered immediately after elevation of salt concentration in the tubular fluid delivered to the JGA and results in the contraction of VSMC of afferent arterioles, thus causing increases in the exposure of proximal tubules to high-salt fluid via the attenuation of glomerular capillary pressure and the glomerular filtration rate (GFR). As a consequence of this negative feedback loop, salt delivery to the distal nephron is kept within a narrow range. This process facilitates the fine adjustment of salt handling in the distal tubules by corticosteroids and peptide hormones, such as aldosterone and arginine vasopressin (AVP).\textsuperscript{42,43}

Similar to TAHL, the apical membrane of the MD cells is abundant in NKCC2, which provides up to 80% of apical NaCl entry in these cells.\textsuperscript{44–46} Early studies of Wilcox and co-workers demonstrated that changes in the plasma [NaCl] affect renal blood flow in dogs mainly via modulation of plasma chloride concentration.\textsuperscript{47,48} It was also shown that regulation of renal blood flow is mediated by activation of [Cl\textsuperscript{−}\textsubscript{o}-sensitive, osmolality-independent TGF.\textsuperscript{49–51} Because of its stoichiometry (1Na\textsuperscript{+}:1K\textsuperscript{+}:2Cl\textsuperscript{−}), the [Cl\textsuperscript{−}\textsubscript{o}] sensing by NKCC2 has an advantage compared to monovalent cations. Indeed, unlike Michael–Menten’s pattern for Na\textsuperscript{+} and K\textsuperscript{+} dependencies, binding of Cl\textsuperscript{−} to NKCC is a cooperative process with a Hill coefficient of 2,\textsuperscript{52} providing high-efficiency regulation of carrier activity in the range of [Cl\textsuperscript{−}\textsubscript{o}] existing in the JGA and close to the EC\textsubscript{50} value of the transporter.
Using isolated rabbit cortical TAHL with attached glomeruli, Laamerti and co-workers determined that NKCC in MD cells was activated by $\mathrm{N}_\mathrm{a}^+$ and $\mathrm{Cl}^-$ with the EC$_{50}$ of 1.0 and 17 mM, respectively.$^{45}$ Importantly, three alternatively-spliced NKCC2 isoforms (A, B and F) cloned from rabbit$^{53}$ and mouse$^{54}$ cDNA libraries. These splice variants, are differently distributed along the nephron: the NKCC2B and the NKCC2A are co-expressed in the MD, whereas the NKCC2F is prevalent in the medullary TAHL.$^{55-57}$ In Xenopus oocytes, the
EC$_{50}$ values of mouse NKCC2B for $\mathrm{K}_o^+$, $\mathrm{N}_\mathrm{a}^+$ and $\mathrm{Cl}^-$ are 0.8, 3.0 and 12 mM, respectively.$^{58}$ Very similar EC$_{50}$ values for $\mathrm{Na}^+$ and $\mathrm{Cl}^-$ were obtained in a study of the rabbit NKCC2B.$^{59}$ In Xenopus oocytes injected with NKCC2A, the EC$_{50}$ values for $\mathrm{Na}^+$ are very close to the those for NKCC2B, whereas the affinity for $\mathrm{Cl}^-$ is 2–5 folds lower.$^{58,59}$ It was also shown that the NKCC2F-transfected oocytes are more energy-efficient in $\mathrm{Na}^+$ uptake thus indicating a key role of this splice-variant in overall salt reabsorption in TAHL.$^{60}$ Indeed, experiments performed in gene knock-out animals demonstrated that NKCC2B and NKCC2A contribute to salt absorption and MD function in the low and high NaCl concentration ranges, respectively.$^{61,62}$ Importantly, the range of NKCC activation by $\mathrm{Cl}^-$ was consistent with the range of modulation of TGF and renin production by the apical NaCl and was similar to the $\mathrm{Cl}^-$ concentration in tubular fluid delivered to rat distal tubules.$^{63}$ Downstream signaling events triggered by acute changes in apical $\mathrm{[Cl]}^-$ were discussed in several comprehensive reviews.$^{2,44,64}$

**Salt secretion by NKCC1**

Unlike apical location of NKCC2 in the absorptive epithelia, NKCC1 was found in the basolateral membrane of the secretory epithelia.$^{65}$ Several laboratories including our team reported that activity of NKCC is decreased by 2–3 folds in erythrocytes from Blacks compared to Caucasians.$^{66-70}$ Keeping in mind that NKCC1 is the only isoforms expressed in erythrocytes, we proposed that attenuated activity of this carrier evokes salt retention seen in African-Americans via its manifestation in the basolateral membrane of sweat glands.$^{71}$ Several observations favour this hypothesis. *First*, both bumetanide-sensitive $^{86}$Rb uptake and chloride fluxes across secretory epithelium are suppressed in NKCC1$^{-/-}$ knock-out mice.$^{72}$ *Second*, in humans, sweat glands contribute to up to 50% of total salt and water excretion during extensive exercise and psychoemotional activity.$^{73}$ *Third*, in a hot environment, the function of sweat glands as a potent regulator of EFV is suppressed in Blacks compared to Caucasians.$^{74}$ *Fourth*, salt retention by sweat glands results in augmented salt loading of renal distal tubule, which, in turn, leads to apical membrane depolarization and enhanced $\mathrm{K}^+$ secretion.$^{75}$ The latter is consistent with more than a 5-fold prevalence of unprovoked hypokalemia detected in African-Americans compared to Caucasians.$^{76}$

In contrast to human, sweat glands have a negligible impact on EFV regulation in rodents and other small mammals.$^{77}$ Because of this, NKCC1$^{-/-}$ knockout mice possessing slightly attenuated blood pressure than wild-type animals$^{72}$ cannot be used to examine the sweat gland-mediated mechanism of NKCC1 involvement in salt handling and hypertension. Keeping this comment in mind, the comparative analysis of $\mathrm{Na}^+$,$\mathrm{K}^+$,$\mathrm{2Cl}^-$ cotransport and transcellular ion transport in secretory epithelial cells from Blacks and Whites is probably
the best approach to examine the relative impact of NKCC1 in blood pressure regulation via its implication in the EFV adjustment.

**Role of NKCC in the pathogenesis of hypertension**

Elevation of systemic blood pressure has been documented in 25% of adults and is a major risk factor for stroke, heart failure, renal disease resulting in premature invalidization and death. A priori, elevation of the systemic blood pressure may be a consequence of the increase of peripheral resistance of blood flow, heart rate and EFV. In turn, above-listed parameters are under the control of dozen of hormones, neurotransmitters and sympathetic nerve system (SNS) affecting heart, blood vessels and renal function. In several forms of systematic hypertension, servomechanisms underlying long-term elevation of blood pressure are well-documented. This is the case of hypertension caused by adrenal tumors and renal insufficiency as well as by a set of single gene mutations in monogenous hypertension and hypotension. These forms of disease found in less than 5% of patients with elevated blood pressure are combined by common name of a secondary hypertension. In the rest of patients with primary or essential hypertension, the mechanisms of blood pressure elevation remain poorly understood.

**Monogenous forms of secondary hypertension and hypotension**

Monogenous forms of hypertension and hypotension identified so far are caused by mutations of genes involved in regulation of EFV by renal epithelial cells. This is consistent with a key role of the kidney in long-term maintenance of elevated blood pressure as described by Artur Guyton. Among monogenous forms of hypertension and hypotension, three types of mutations result in altered function of CCCs. The loss-of-function mutations of NKCC2 and NCC detected in patients with Barter type I and Gitelman syndrome, respectively, lead to attenuated salt reabsorption in thick ascending limb of Henle’s loop and distal nephron, which, in turn, results in a decreased EFV and systemic blood pressure. In both diseases inherited in accordance with classic Mendel’s genetics, hypotension is accompanied by hypokalemia and alkaloidosis – universal markers of decreased reabsorption of salt in distal nephron. In contrast, in patients with pseudohypoaldosteronism type II (PHAII) also known as Gordon’s syndrome, hypertension is caused by mutations in with-no-K(lysine) kinases WNK1 and WNK4 resulting in activation of NCC, increased sodium reabsorption and hyperkalemia.

**Primary hypertension**

Unlike monogenous forms of secondary hypertension, elevation of blood pressure in primary hypertension is a consequence of a complex combination of inheriting traits and several environmental factors including limited physical activity, obesity, smoking, the augmented consumption of salt and alcohol. Inherited traits are probably caused by altered function of 4–5 genes whose combination may be different even within the same human population, underlining mosaic origin of the pathogenesis of essential hypertension as noted for the first time by Pickering. Upto now, there are no reports on mutations of NKCC2 and other renal-specific CCCs in patients with essential hypertension. Schiebl and co-workers found that in mice differential splicing of NKCC2 pre-mRNA is modulated by dietary salt intake, which may be triggered by recently discovered [Na+]i/[K+]i-mediated
excitation-transcription coupling (for review, see\textsuperscript{88}). Enhanced tubular reabsorption of salt and osmotically-obliged water is an important determinant of obesity-related hypertension. Davies and co-workers reported that increased activity of NKCC2 in high-fat diet mice is caused by STE20/SPS1-related proline/alanine-rich kinase SPAK/OSR1-mediated phosphorylation of this carrier at serine-126.\textsuperscript{89} Additional studies should be performed to examine the role of this signaling pathway in the regulation of renal-specific CCCs in the pathogenesis of essential hypertension.

In the early studies, it was shown that elevated permeability for monovalent cations of VSMC\textsuperscript{90} as well as erythrocytes from spontaneously hypertensive rats (SHR)\textsuperscript{91} and patients with essential hypertension\textsuperscript{92} is at least partially caused by augmented activity of NKCC (for review, see.\textsuperscript{93–97}). Because NKCC1 is the only isoform of Na\textsuperscript{+}, K\textsuperscript{+},2Cl\textsuperscript{−} cotransporters identified in erythrocytes, these data indicate that at least in these experimental models of human primary hypertension, augmented activity of this carrier contributes to activation of servomechanisms for long-term elevation of systemic blood pressure. This conclusion can be supported by several observations. 

\textit{First}, in erythrocytes of F1 hybrids obtained by crossing of Milan hypertensive strain (MHS) and Milan normotensive strain (MNS) rats and subjected to X-ray irradiation, NKCC activity was increased after transplantation of bone marrow from MHS but not from MNS.\textsuperscript{98} \textit{Second}, in erythrocytes of F\textsubscript{2} MHS × MNS hybrids as well as F2 hybrids obtained by crossing of SHR and normotensive Wistar-Kyoto (WKY) rats, NKCC activity positively correlated with blood pressure.\textsuperscript{98,99} \textit{Third}, several researchers demonstrated decreased blood pressure in NKCC1\textsuperscript{−/−} knock out mice.\textsuperscript{72,100,101} \textit{Fourth}, administration of bumetanide, a potent inhibitor of Na\textsuperscript{+}, K\textsuperscript{+},2Cl\textsuperscript{−} cotransport, decreased blood pressure in wild-type but not in NKCC1\textsuperscript{−/−} mice.\textsuperscript{27}

These findings raise a question on the mechanisms for the involvement of NKCC1 in the pathogenesis of hypertension. Data considered above suggest that these mechanisms may involve NKCC1-mediated regulation of [Cl\textsuperscript{−}]\textsubscript{i} which, in turn, affects VSMC contraction and SNS activity. Indeed, it was shown that inhibitory action of bumetanide on the contraction of mesenteric arteries evoked by activation of \(\alpha\)-adrenergic receptor is increased in SHR compared to normotensive controls.\textsuperscript{102,103} Due to the methodological problems, data on the activity of NKCC in freshly isolated VSMC in primary hypertension are limited to few publications.\textsuperscript{94,95} It was shown, however, that the content of NKCC1 mRNA and immunoreactive protein is increased in aorta and heart from SHR.\textsuperscript{102} Elevation of sympathetic tone may lead to elevation of systemic blood pressure via its impact on cardiovascular system and the kidney.\textsuperscript{104–107} This hypothesis is consistent with numerous reports on activation of SNS in patients with essential hypertension\textsuperscript{104} and SHR.\textsuperscript{108}

The major role of CNS in activation of paraventricular nucleus (PVN) in hypothalamus possessing hyperactivity in primary hypertension is well documented.\textsuperscript{109–111} It is also known that presynaptic neurons in PVN are activated by excitatory glutamatergic neurones and suppressed by inhibitory GABAergic neurons, respectively.\textsuperscript{112} Importantly, activity of GABAergic neurons is decreased in PVN of SHR.\textsuperscript{112,113} As mentioned above, the relationship between the inhibitory and stimulatory actions of GABA\textsubscript{A} receptors is determined by intracellular chloride concentration that is under the control of the ratio of NKCC1 vs. KCC2 activities. Recent studies demonstrated that the value of the equilibrium
potential for GABA<sub>A</sub> receptors (E<sub>GABA</sub>) is shifted to positive values in PVN of SHR by 15 mV, which corresponds to a 2-fold elevation of [Cl<sup>-</sup>]<sub>i</sub> compared to normotensive control. This difference as well as decreased inhibitory activity of GABAergic neurons in SHR was abolished by addition of low doses of bumetanide but not furosemide. These results allowed authors to propose that augmented [Cl<sup>-</sup>]<sub>i</sub> in SHR neurons is caused by activation of NKCC1 rather than inhibition of KCC2. This conclusion is in agreement with elevated of NKCC1 mRNA and immunoreactive protein levels with no changes in KCC2 content in PVN of SHR.

Mechanisms of elevation of NKCC1 activity in primary hypertension remain poorly investigated, which probably reflects the polygenic and mosaic origin of this disease as well as diverse mechanisms of regulation of the activity and expression of CCCs. For example, elevation of [Ca<sup>2+</sup>]<sub>i</sub> activates whereas production of cAMP inhibits this carrier in VSMC. Numerous investigations documented abnormal activity of both signaling systems in primary hypertension. Recent studies demonstrated a key role of WNK, SPAK and OSR1 kinases in regulation of several CCCs including NKCC1 and NKCC2 (for reviews, see). Bergaya and co-workers reported that both phosphorylation of NKCC1 and increment of blood pressure evoked by activation of α-adrenergic receptors is decreased in Wnk<sup>+/−</sup> mice. Bumetanide-sensitive component of vessel contraction was also attenuated in SPAK knock-out mice. To further examine the role of this signalling pathway, Rafiqi and co-workers generated knock-in mice in which SPAK cannot be activated by WNKs. These animals display reduced salt-dependent increment of blood pressure as well as decreased expression of NKCC2 and NCC proteins with no changes in mRNA level. It is noteworthy, however, that there is no evidence for mutations of genes encoding CCCs or WNK/SPAK/OSR1 regulatory pathway in primary hypertension in contrast to monogenic hypertension.

Epigenetic phenomena include DNA methylation, post-translation histone modification and expression of non-coding RNAs. Epigenetic mechanisms are under complex control of diverse environmental factors, which make them crucial for their involvement in the pathogenesis of hypertension and other complex and multifactorial disorders. Recent studies suggest that NKCC1-mediated abnormalities of ion transport have epigenetic origin. It has been shown that the contents of NKCC1 mRNA and protein are increased in aorta, heart and PVN neurones from rats with spontaneous hypertension. In SHR aorta and heart, increased expression of NKCC1 is accompanied by attenuated methylation of its promoter. Importantly, methylation of NKCC1 promoter exhibited age-dependent increase in normotensive rats but not in SHR. It was also shown that the activity of DNA methyl-transferase 3B (DNTB3B) is 3-fold higher in normotensive rats as compared to age-matched SHR. These results suggest that in this experimental model of primary hypertension, decreased activity of DNTB3B results in hypo-methylation of NKCC1 promoter, which, in turn, leads to augmented NKCC1 expression, increment of [Cl<sup>-</sup>]<sub>i</sub>, depolarization and contraction of SMC, increased vascular resistance and elevation of blood pressure.

The role of epigenetic factors in augmented expression of NKCC1 in neurones of PVN of SHR, controlling activity of SNS, remains unknown. However, it was shown that in PVN
from SHR, NKCC1 is highly glycosylated, which may contribute to increased content of membrane-bound protein, i.e., the fraction of the carrier providing Na\(^+\), K\(^+\),2Cl\(^-\) cotransport.\(^{114}\) Thus, glycosylation of NKCC could represent another level of its regulation.

**Complications caused by elevated blood pressure**

The major cause of the premature death of patients with essential hypertension is a damage of the target organs such as brain vessels and the kidney due to chronic elevation of local blood pressure.\(^{128}\) Blood pressure elevation in the brain microcirculation increases the probability of nonreversible damage of the blood flow that results in stroke, whereas in the kidney high blood pressure leads to structural alterations in the nephron, changes of salt-water homeostasis and proteinuria.\(^{78}\)

Keeping in mind that \(R_{bf} \approx 1/d^4\) (where \(R_{bf}\) is the blood flow resistance and \(d\) is the inner vessel’s diameter),\(^{129}\) myogenic response plays a key role in the protection of target organs from elevation of systemic arterial pressure. It was shown that chronic suppression of myogenic response in patients with essential hypertension as a consequence of hypertrophy of vessel’s wall leads to attenuation of its sensitivity to the changes of intraluminal pressure. As a result of these changes, an increment of systemic blood pressure is transferred to microcirculation beds incorporated into the brain, heart, retina, kidney, which causes irreversible changes in the structure and function of these and other target-organs of hypertension.\(^{130,131}\)

To investigate the role of myogenic response in the kidney function, Loutzenhiser and co-workers employed isolated perfused kidney, which allowed them to study renal microcirculation in the absence of its modulation by JGA.\(^{132}\) Using this model, they have shown that bumetanide completely suppresses myogenic response of afferent arteriole in the rat kidney.\(^{23}\) Together with the absence of myogenic response in \(Nkcc1^{-/-}\) mice\(^{33}\), these results may suggest that augmented activity of NKCC1 in SHR, MHS rats and in patients with essential hypertension protects kidney from the damage by prolonged elevation of the systemic blood pressure whereas chronic administration of furosemide and other NKCC inhibitors accelerates the renal insufficiency and proteinuria.\(^{71,96,97}\) In other words, high activity of NKCC1 in VSMC of the afferent arteriole maintains the constant renal blood flow even after elevation of systemic blood pressure caused by activation of this carrier in mesenteric arteries and other vessels contributing to regulation of peripheral resistance (Fig. 1). This hypothesis is consistent with 4-fold increase of renal complications in African-Americans with hypertension possessing up to 3-fold attenuation of NKCC activity in erythrocytes as compared to age-matched hypertensive Caucasians.\(^{70,71}\) The relative contribution of Ca\(^{2+}\) channels and NKCC1 in the myogenic response of coronary and SNS microcirculatory beds remains unexplored.

Ischemia is the major consequence of even a brief disturbance of blood circulation in the brain vessels that leads to irreversible damage of the neuronal function. It was shown that attenuation of the oxygen partial pressure results in astrocyte swelling, which in turn leads to a release of glutamate and other neurotransmitters triggering massive Ca\(^{2+}\) influx in neurones and their death.\(^{133,134}\) It was shown that bumetanide suppresses astrocyte swelling, neurotransmitter’s release,\(^{135}\) and the death of neurones in the hippocampus subjected to
hypoxia and hypoglycemia.\textsuperscript{136} Moreover, both [K\textsuperscript{+}]\textsubscript{i}-induced swelling and release of neurotransmitters evoked by ischemia are sharply decreased in astrocytes from NKCC1\textsuperscript{−/−} mice,\textsuperscript{137} thus indicating that activation of NKCC1 is a mechanism for astrocyte swelling.

We have reported that elevation of [HCO_3\textsuperscript{−}]\textsubscript{i} decreases NKCC activity in VSMC from rat aorta.\textsuperscript{138} Thus, activation of NKCC1 under hypoxic conditions might be caused by acidosis-mediated attenuation of [HCO_3\textsuperscript{−}] in cerebrospinal fluid. Hypoxia is also accompanied by sharp attenuation of intracellular ATP content and inhibition of the Na\textsuperscript{+},K\textsuperscript{+}-ATPase.\textsuperscript{139} As shown in Table 2, inhibition of the Na\textsuperscript{+},K\textsuperscript{+}-ATPase changes the direction of NKCC1-mediated net ion fluxes and evokes cell swelling. Additional experiments should be performed for evaluation of the relative impact of Na\textsuperscript{+},K\textsuperscript{+}-pump and NKCC1 in regulation of astrocyte volume in ischemic conditions.

**Conclusion**

The data discussed in this review leads to several conclusions.

*First*, the transport of monovalent ions across the cells as well as the regulation of intracellular chloride concentration and of cell volume are major functions of NKCCs. In renal epithelial cells, reabsorption of salt and osmotically-obliged water is provided by NKCC2, whereas NKCC1 has a major impact on [Cl\textsuperscript{−}]\textsubscript{i} in VSMC and neuronal cells.

*Second*, in primary hypertension, NKCC1 activity is increased in VSMC and neurons of PVN leading to elevation of peripheral resistance in systemic circulation and activation of SNS, respectively. In both cases, these abnormalities are mediated by elevation of [Cl\textsuperscript{−}]\textsubscript{i} and plasma membrane depolarization.

*Third*, furosemide and other loop’s diuretics decrease systemic blood pressure via inhibition of NKCC2 in TAHL and of NKCC1 in smooth muscles of resistant vessels. However, the same compounds suppress the myogenic response in microcirculatory beds of the kidney and the brain thus increasing the risk of renal and cerebral complications.

In spite of the progress in the understanding the role of NKCC in the regulation of cellular functions at physiological and pathophysiological conditions, several questions remain unanswered. In cultured VSMC as well as in isolated blood vessels, NKCC1 is activated by phenylephrine, angiotensin II and other vasoconstrictors and is inhibited by vasodilators whose action is mediated by cAMP.\textsuperscript{26,115} Recently, Danielsson and co-workers have reported that the combination of Cl-channel blockers and bumetanide potentiates relaxation of airway smooth muscle rings by β-adrenergic agonists.\textsuperscript{140} Hence, the question is whether NKCC1 contributes to the regulation of vascular tone by these compounds. A key role of NKCC1 in regulation of myogenic response of renal afferent arteriole is well-documented. The question is what is the relative contribution of NKCC1 to the regulation of myogenic response of microcirculatory beds of the brain and other target-organs of hypertension.

*In vitro* experiments have demonstrated that loop’s diuretics might be used as a pharmacological tools augmenting inhibitory function of GABA\textsubscript{A} receptors. It was shown that at least furosemide fluently penetrates human blood–brain barrier.\textsuperscript{141} This finding
together with the data discussed in the previous sections suggests that high-ceiling diuretics may be used for the regulation of sympathetic tone via inhibition of NKCC1 in neuronal cells. Indeed, it was shown that orally administrated furosemide at doses commonly used in clinic reduced spinal inhibitory inter-neuronal activity. It is important to point out that currently used high-ceiling diuretics exhibit the same affinity for NKCC1 and NKCC2. Because an apparent affinity for furosemide and bumetanide is proportional to carrier’s activity, inhibition of highly active NKCC2 and diuretic action of these compounds is much greater than their vasodilatory and neuronal effects. It is also important to mention another side effect of these drugs, i.e., their prolonged administration may result in the development of deafness due to inhibition of NKCC1 in epithelial cells of the inner ear. These issues become important for the development of novel antihypertensive drugs lacking side-effects caused by NKCC inhibition in epithelial cells and myogenic tonus in microcirculatory beds.

Acknowledgments

This work was supported by grants from the Canadian Institutes for Health Research (MOP-81392) (S.N.O.), Russian Foundation for Fundamental Research ##14-04-31705 (S.V.K.), 15-04-00101 (S.N.O.), the Russian Scientific Foundation #14-15-00006 (S.N.O.) and the USA National Institutes of Health Award R01-GM85058 (N.O.D.).

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| CCC          | cation-chloride cotransporters |
| CNS          | central nervous system |
| EFV          | extracellular fluid volume |
| GABA         | γ-aminobutiric acid |
| GFR          | glomerular filtration rate |
| JGA          | juxtaglomerular apparatus |
| KCC          | K⁺,Cl⁻ cotransport |
| MD           | macula densa |
| NCC          | Na⁺,Cl⁻ cotransport |
| NKCC         | Na⁺,K⁺,2Cl⁻ cotransport |
| OSR1         | oxidative stress response kinase |
| PVN          | paraventricular nucleus |
| SMC          | smooth muscle cells |
| SNS          | sympathetic nervous system |
| SPAK         | Ste20-related praline-alanine-rich kinase |
| TAHL         | thick ascending limb of Henle’s loop |
| TGF          | tubuloglomerular feedback |
WNK with no K lysine kinase

References

1. Gamba G. Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters. Physiol Rev. 2005; 85:423–493. [PubMed: 15788703]

2. Orlov SN, Mongin AA. Salt sensing mechanisms in blood pressure regulation and hypertension. Am J Physiol Heart Circ Physiol. 2007; 293:H2039–H2053. [PubMed: 17693546]

3. Markadieu N, Delpire E. Physiology and pathophysiology of SLC12A1/2 transporters. Pfluger Arch - Eur J Physiol. 2014; 466:91–105.

4. Lang F, Busch G, Ritter M, et al. Functional significance of cell volume regulatory mechanisms. Physiol Rev. 1998; 78:247–306. [PubMed: 9457175]

5. Mongin AA, Orlov SN. Mechanisms of cell volume regulation and possible nature of the cell volume sensor. Pathophysiology. 2001; 8:77–88. [PubMed: 11720802]

6. Orlov SN, Pokudin NI, Kotelevtsev YuV, Gulak PV. Volume-dependent regulation of ion transport and membrane phosphorylation in human and rat erythrocytes. J Memb Biol. 1989; 107:105–117. [PubMed: 2541247]

7. Adragna N, Di Fulvio M, Lauf PK. Regulation of K-Cl cotransport: from function to genes. J Memb Biol. 2004; 201:109–137. [PubMed: 15711773]

8. Orlov SN. Ion transport across erythrocyte membrane: mechanisms and volume-dependent regulation. Sov Sci Rev F Physiol Gen Biol. 1994; 8:1–48.

9. Orlov SN, Tremblay J, Hamet P. Cell volume in vascular smooth muscle is regulated by bumetanide-sensitive ion transport. Am J Physiol. 1996; 270:C1388–C1397. [PubMed: 8967439]

10. Hoffmann EK, Lambert IH, Pedersen SF. Physiology of cell volume regulation in vertebrates. Physiol Rev. 2009; 89:193–277. [PubMed: 1912658]

11. Orlov SN, Platonova AA, Hamet P, Grygorczyk R. Cell volume and monovalent ion transporters: their role in the triggering and progression of the cell death machinery. Am J Physiol Cell Physiol. 2013; 305:C361–C372. [PubMed: 23615964]

12. Alvarez-Leefmans, FJ. Intracellular chloride regulation. In: Sperelakis, N., editor. Cell Physiology Source Book. A Molecular Approach. San Diego, CA: Academic; 2001. p. 301-318.

13. Chipperfield AR, Harper AA. Chloride in smooth muscle. Prog Biophys Mol Biol. 2001; 74:175–221. [PubMed: 11226512]

14. Davis JPL, Chipperfield AR, Harper AA. Accumulation of intracellular chloride by (Na-K-Cl) cotransport in rat arterial smooth muscle is enhanced in deoxycorticosterone acetate (DOCA)/salt hypertension. J Mol Cell Cardiol. 1993; 25:233–237. [PubMed: 8510166]

15. Anfinogenova YJ, Baskakov MB, Kovalev IV, Kilin AA, Dulin NO, Orlov SN. Cell-volume-dependent vascular smooth muscle contraction: role of Na+,K+,2Cl− cotransport, intracellular Cl− and L-type Ca2+ channels. Pflugers Arch. 2004; 449:42–55. [PubMed: 15293051]

16. Barthelmebs M, Stephan D, Fontaine C, Grima M, Imbs JL. Vascular effects of loop diuretics: an in vivo and in vitro study in the rat. Schmiedeb Arch Pharmacol. 1994; 349:209–216.

17. Lavallee SL, Iwamoto LM, Claybaugh JR, Dressel MV, Sato AK, Nakamura KT. Furosemide-induced airway relaxation in guinea pigs: relation to Na-K-2Cl cotransporter function. Am J Physiol. 1997; 273:L211–L216. [PubMed: 9252558]

18. Tian R, Aalkjaer C, Andreasen F. Mechanisms behind the relaxing effect of furosemide on the isolated rabbit ear artery. Pharmacol Toxicol. 1990; 67:406–410. [PubMed: 2096384]

19. Kovalev IV, Baskakov MB, Anfinogenova YJ, et al. Effect of Na+,K+,2Cl− cotransport inhibitor bumetanide on electrical and contractile activity of smooth muscle cells in guinea pig ureter. Bull Exp Biol Med. 2003; 136:145–149. [PubMed: 14631495]

20. Kovalev IV, Baskakov MB, Medvedev MA, et al. Na+,K+,2Cl−-cotransport and chloride permeability of the cell membrane in mezaton and histamine regulation of electrical and contractile activity in smooth muscle cells from the guinea pig ureter. Russ Physiol J. 2008; 93:306–317.
21. Stanke F, Devillier P, Breant D, et al. Furosemide inhibits angiotensin II-induced contraction on human vascular smooth muscle. Br J Clin Pharmacol. 1998; 46:571–575. [PubMed: 9862246]
22. Stanke-Labesque F, Craciwski JL, Bedouch P, et al. Furosemide inhibits thrombaxane A2-induced contraction in isolated human internal artery and saphenous vein. J Cardiovasc Pharmacol. 2000; 35:531–537. [PubMed: 1077481]
23. Wang X, Breaks J, Loutzenhis K, Loutzenhis R. Effects of inhibition of the Na+/K+/2Cl- cotransporter on myogenic and angiotensin II responses of the rat afferent arteriole. Am J Physiol Ren Physiol. 2007; 292:F999–F1006.
24. Mozhayeva MG, Bagrov YY. The inhibitory effects of furosemide on Ca2+ influx pathways associated with oxytocin-induced contractions of rat myometrium. Gen Physiol Biophys. 1995; 14:427–436. [PubMed: 8786042]
25. Mozhayeva MG, Bagrov YY, Ostretsova IB, Gillespie JI. The effect of furosemide on oxytocin-induced contractions of the rat myometrium. Exp Physiol. 1994; 79:661–667. [PubMed: 781857]
26. Akar F, Skinner E, Klein JD, Jena M, Paul RJ, O’Neill WC. Vasoconstrictors and nitrovasodilators reciprocally regulate the Na+-K+-2Cl- cotransporter in rat aorta. Am J Physiol. 1999; 276:C1383–C1390. [PubMed: 10362601]
27. Garg P, Martin C, Elms SC, et al. Effect of the Na-K-2Cl cotransporter NKCC1 on systemic blood pressure and smooth muscle tone. Am J Physiol Heart Circ Physiol. 2007; 292:H2100–H2105. [PubMed: 17259435]
28. Palacios J, Espinoza F, Munita C, Cifuentes F, Michea L. Na+-K+-2Cl- cotransporter is implicated in gender differences in the response of the rat aorta to phenylephrine. Br J Pharmacol. 2006; 148:964–972. [PubMed: 1679647]
29. Koltsova SV, Maximov GV, Kotelevtsev SV, et al. Myogenic tone in mouse mesenteric arteries: evidence for P2Y receptor-mediated, Na+,K+-2Cl- cotransport-dependent signaling. Purinergic Signal. 2009; 5:343–349. [PubMed: 19387869]
30. Davis MJ, Hill MA. Signaling mechanisms underlying the vascular myogenic response. Physiol Rev. 1999; 79:387–423. [PubMed: 10221985]
31. Hill MA, Davis MJ, Meininger GA, Potocnik SJ, Murphy TV. Arteriolar myogenic signaling mechanisms: implications for local vascular functions. Clin Hemorheol Microcirc. 2006; 34:67–79. [PubMed: 16543619]
32. Schubert R, Mulvany MJ. The myogenic response: established facts and attractive hypothesis. Clin Sci. 1999; 96:313–326. [PubMed: 10087237]
33. Koltsova SV, Kotelevtsev SV, Tremblay J, Hamet P, Orlov SN. Excitation-contraction coupling in resistant mesenteric arteries: evidence for NKCC1-mediated pathway. Biochem Biophys Res Commun. 2009; 379:1080–1083. [PubMed: 19150334]
34. Delipire E, Austin TM. Kinase regulation of Na+-K+-2Cl-cotransport in primary neurons. J Physiol. 2010; 588:3365–3373. [PubMed: 20498230]
35. Kahle KT, Staley KJ, Nahed BV, et al. Roles of the cation-chloride cotransporters in neurological disease. Nat Clin Pract Neurol. 2008; 4:490–503. [PubMed: 1876937]
36. Loscher W, Puskarjov M, Kaila K. Cation-chloride cotransporters NKCC1 and KCC2 as potential targets for novel anti-epileptic and antiepileptogenic treatments. Neuropsychopharmacology. 2013; 69:62–74. [PubMed: 22705273]
37. Wright FS, Scherrnann J. Interference with feedback control of glomerular filtration rate by furosemide, triflucin, and cyanide. J Clin Invest. 1974; 53:1695–1708. [PubMed: 4830232]
38. Simon DB, Karet FE, Hamdan JM, Di Pietro A, Sanjad SA, Lifton RP. Bartter’s syndrome, hypokalemic alkalosis with hypercalciuria, is caused by mutation of Na-K-2Cl cotransporter NKCC2. Nat Genet. 1996; 13:183–188. [PubMed: 8640224]
39. Briggs, JP.; Schnermann, J. Control of renin release and glomerular vascular tone by the juxtaglomerular apparatus. In: Laragh, JH.; Brenner, BM., editors. Hypertension: Pathophysiology, Diagnosis, and Management. 2. New York: Raven Press Ltd; 1995. p. 1359-1384.
40. Scherrmann J, Briggs JP. Tubuloglomerular feedback: mechanistic insights from gene-manipulated mice. Kidney Int. 2008; 74:418–426. [PubMed: 18418352]
41. Singh P, Thomson SC. Renal homeostasis and tubuloglomerular feedback. Curr Opin Nephrol Hypert. 2010; 19:59–64.
42. Knepper MA, Inoue T. Regulation of aquaporin-2 water channel trafficking by vasopressin. Curr Opin Cell Biol. 1997; 9:560–564. [PubMed: 9261056]
43. Borgnia M, Nielsen S, Engel A, Agre P. Cellular and molecular biology of the aquaporin water channels. Annu Rev Biochem. 1999; 68:425–458. [PubMed: 10872456]
44. Bell PD, Lapointe J-Y, Peti-Peterdi J. Macula densa cell signaling. Annu Rev Physiol. 2003; 65:481–500. [PubMed: 12524458]
45. Laamarti MA, Bell PD, Lapointe J-Y. Transport and regulatory properties of the apical Na-K-2Cl cotransporter of macula densa cells. Am J Physiol. 1998; 275:F703–F709. [PubMed: 9815128]
46. Nielsen S, Maunsbach AB, Ecelbarger CA, Knepper MA. Ultra-sructural localization of Na-K-2Cl cotransporter in thick ascending limb and macula densa of rat kidney. Am J Physiol. 1998; 275:F885–F893. [PubMed: 9843905]
47. Nashat FS, Tappin JW, Wilcox CS. The renal blood flow and the glomerylar filtration rate of anaesthetized dogs during acute changes in plasma sodium concentration. J Physiol. 1976; 256:731–745. [PubMed: 5603]
48. Wilcox CS. Regulation of renal blood flow by plasma chloride. J Clin Invest. 1983; 71:726–735. [PubMed: 6826732]
49. Briggs JP, Schermann J, Wright FS. Failure of tubule fluid osmolarity to affect feedback regulation of glomerular filtration. Am J Physiol. 1980; 239:F427–F432. [PubMed: 6776824]
50. Schnerrnann J, Briggs J, Wright FS. Feedback-mediated reduction of glomerular filtration rate during infusion of hypertonic saline. Kidney Int. 1981; 20:462–468. [PubMed: 7311307]
51. Schnerrnann J, Ploth DW, Hermle M. Activation of tubulo-glomerular feedback by chloride transport. Pflugers Arch. 1976; 362:229–240. [PubMed: 944430]
52. Russell JM. Sodium-potassium-chloride cotransport. Physiol Rev. 2000; 80:212–276.
53. Payne JA, Forbush B. Alternatively spliced isoforms of the putative renal Na-K-Cl cotransporter are differently distributed within the rabbit kidney. Proc Natl Acad Sci U S A. 1994; 91:4544–4548. [PubMed: 7514306]
54. Igarashi P, Vandel Heuvel GB, Payne JA, Forbush BI. Cloning, embryonic expression, and alternative splicing of a murine kidney-specific Na-K-Cl cotransporter. Am J Physiol. 1995; 269:F405–F418. [PubMed: 7573490]
55. Castrop H, Schnermann J. Isoforms of renal Na-K-2Cl cotransporter NKCC2: expression and functional significance. Am J Physiol Ren Physiol. 2008; 295:F859–F866.
56. Yang T, Huang YC, Singh I, Schnermann J, Briggs JP. Localization of bumetanide- and thiazide-sensitive Na-K-Cl cotransporters along the rat nephron. Am J Physiol. 1998; 271:F931–F939. [PubMed: 8898025]
57. Brunet GM, Gagnon E, Simard CF, et al. Novel insights regarding the operational characteristics and theological purpose of the renal Na+,K+,2Cl- cotransporter (NKCC2s) splice variants. J Gen Physiol. 2005; 126:325–337. [PubMed: 16157691]
58. Plata C, Meade P, Vazquez N, Hebert SC, Gamba G. Functional properties of the apical Na+,K+,2Cl- cotransporter isoforms. J Biol Chem. 2002; 277:11004–11012. [PubMed: 11790783]
59. Gimenez I, Isenring P, Forbush BI. Spatially distributed alternatively spliced variants of the renal Na-K-Cl cotransporter exhibit dramatically different affinities for the transported ions. J Biol Chem. 2002; 277:8767–8770. [PubMed: 11815599]
60. Lu L, Fraser JA. Functional consequences of NKCC2 splice isoforms: insight from a Xenopus oocyte model. Am J Physiol Ren Physiol. 2014; 306:F710–F720.
61. Oppermann M, Mizel D, Huang G, et al. Macula densa control of renin secretion and proglomerular resistance in mice with selective deletion of the B isoform of Na, K,2Cl co-transporter. J Am Soc Nephrol. 2006; 17:2143–2152. [PubMed: 16807402]
62. Oppermann M, Mizel D, Kim SM, et al. Renal function in mice with targeted disruption of the A isoform of the Na-K-2Cl- co-transporter. J Am Soc Nephrol. 2007; 18:440–448. [PubMed: 17215439]
63. Lorenz JN, Kotchen TA, Ott CE. Effect of Na and Cl infusion on loop function and plasma renin activity. Am J Physiol. 1990; 258:F1328–F1335. [PubMed: 2186635]

64. Peti-Peterdi J, Bebok Z, Lapointe J-Y, Bell PD. Cytosolic [Na] regulation in macula densa cells: novel role for an apical H-K-ATPase. Am J Physiol Ren Physiol. 2002; 282:F329.

65. O’Grady SM, Pulfrey HC, Field M. Characteristics and functions of Na-K-Cl cotransport in epithelial tissues. Am J Physiol. 1987; 253:C177–C192. [PubMed: 3303961]

66. Tuck ML, Gross C, Maxwell MH, Brickman AS, Krasnoshtein G, Mayes D. Erythrocyte Na⁺,K⁺ cotransport and Na⁺,K⁺ pump in black and caucasian hypertensive patients. Hypertension. 1984; 6:536–544. [PubMed: 6086157]

67. Weder AB, Torretti BA, Julius S. Racial differences in erythrocyte cation transport. Hypertension. 1984; 6:115–123. [PubMed: 6693141]

68. Canessa M. The Na-K-Cl cotransport in essential hypertension: cellular functions and genetic environment interactions. Int J Cardiol. 1989; 25:S37–S45. [PubMed: 2695469]

69. Orlov SN, Pausova Z, Gossard F, et al. Na⁺,K⁺,Cl⁻ cotransport is decreased in African American vs French-Canadian hypertensives: lack of impact of gender and plasma lipids (Abstract). J Hypertens. 2001; 19(suppl 2):S46.

70. Orlov SN, Gossard F, Pausova Z, et al. Decreased NKCC1 activity in erythrocytes from African-Americans with hypertension and dyslipidemia. Am J Hypertens. 2010; 23:321–326. [PubMed: 20044742]

71. Orlov SN. Decreased Na⁺,K⁺,Cl⁻ cotransport and salt retention in Blacks: a provocative hypothesis. J Hypertens. 2005; 23:1929–1930. [PubMed: 16148618]

72. Flagella M, Clarke LL, Miller ML, et al. Mice lacking the basolateral Na-K-2Cl cotransporter have impaired epithelial chloride secretion and are profoundly deaf. J Biol Chem. 1999; 274:26946–26955. [PubMed: 10480906]

73. Saito T, Hartell NA, Muguruma H, Hotta S, Sasaki S, Ito M. Light dose and time dependency of photodynamic cell membrane damage. Photochem Photobiol. 1998; 68:745–748. [PubMed: 9825704]

74. Dill DB, Yousef MK, Goldman A, Hillyard SD, Davis TP. Volume and composition of hand sweat of white and black men and women in desert walk. Am J Antropol. 1983; 61:67–73.

75. Giebisch G. Renal potassium transport: mechanisms and regulation. Am J Physiol. 1998; 274:F817–F833. [PubMed: 9612319]

76. Andrew PM, Jones DW, Wofford MR, et al. Ethnicity and unprovoked hypokalemia in the atherosclerosis risk in communities study. Am J Hypertens. 2002; 15:594–599. [PubMed: 12118905]

77. Sato K, Kang WH, Saga K, Sato KT. Biology of sweet glands and their disorders. I. Normal sweat gland function. J Am Acad Dermatol. 1989; 20:537–563. [PubMed: 2654204]

78. O’Shaughnessy KM, Karet FE. Salt handling in hypertension. Annu Rev Nutr. 2006; 26:343–365. [PubMed: 16602929]

79. Guyton, AC. Arterial Pressure and Hypertension. Philadelphia: WB Saunders Co; 1980.

80. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell. 2001; 104:545–556. [PubMed: 11239411]

81. Lifton RP. Genetic dissection of human blood pressure variation: common pathways from rare phenotypes. Harvey Lect. 2005; 100:71–101. [PubMed: 16970175]

82. Guyton AC. Blood pressure control – special role of the kidney and body fluids. Science. 1991; 252:1813–1816. [PubMed: 2063193]

83. Simon DB, Nelson-Williams C, Bia J, et al. Gitelman’s variant of Bartter’s syndrome, inherited hypokalemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. Nat Genet. 1996; 12:24–30. [PubMed: 8528245]

84. Wilson FH, Disse-Nicodeme S, Choate KA, et al. Human hypertension caused by mutations in WNK kinases. Science. 2001; 293:1107–1112. [PubMed: 11498583]

85. Hamel P, Pausova Z, Adarichev V, Adaricheva K, Tremblay J. Hypertension: genes and environment. J Hypertens. 1998; 16:397–418. [PubMed: 9797185]

Genes Dis. Author manuscript; available in PMC 2015 June 23.
86. Pickering, GW. Systematic arterial pressure. In: Fishman, AP.; Richards, DW., editors. Circulation of the Blood. Men and Ideas. London: 1964. p. 487-541.

87. Schiebl IM, Rosenauer A, Kattler V, Minuth WW, Oppermann M, Castrop H. Dietary salt intake modulates differential splicing of the Na-K-2Cl cotransporter NKCC2. Am J Physiol Ren Physiol. 2013; 305:F1139–F1148.

88. Orlov SN, Hamet P. Salt and gene expression: evidence for Na⁺,K⁺-mediated signaling pathways. Pfluger Arch – Eur J Physiol. 2015; 467:475–487.

89. Davies M, Fraser SA, Galic S, et al. Novel mechanisms of Na⁺ retention in obesity: phosphorylation of NKCC2 and regulation of SPAK/OSR1 by AMPK. Am J Physiol Ren Physiol. 2014; 307:F96–F106.

90. Jones AW. Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats. Influence of aldosterone, norepinephrine and angiotensin. Circ Res. 1973; 33:563–572. [PubMed: 4356609]

91. Postnov, YuV; Orlov, SN.; Gulak, PV.; Shevchenko, AS. Altered permeability of the erythrocyte membrane for sodium and potassium in spontaneously hypertensive rats. Pflugers Arch. 1976; 365:257–263. [PubMed: 988566]

92. Postnov, YuV; Orlov, SN.; Shevchenko, AS.; Adler, AM. Altered sodium permeability, calcium binding and Na-K-ATPase activity in the red blood cell membrane in essential hypertension. Pflugers Arch. 1977; 371:263–269. [PubMed: 146188]

93. Postnov, YuV; Orlov, SN. Ion transport across plasma membrane in primary hypertension. Physiol Rev. 1985; 65:904–945. [PubMed: 2414791]

94. Orlov SN, Adragna N, Adarichev VA, Hamet P. Genetic and biochemical determinants of abnormal monovalent ion transport in primary hypertension. Am J Physiol. 1999; 276:C511–C536. [PubMed: 10069978]

95. Garay RP, Alda O. What can we learn from erythrocyte Na-KCl cotransporter NKCC1 in human hypertension. Pathophysiology. 2007; 14:167–170. [PubMed: 17942286]

96. Orlov SN, Tremblay J, Hamet P. NKCC1 and hypertension: a novel therapeutic target involved in regulation of vascular tone and renal function. Curr Opin Nephrol Hypert. 2010; 19:163–168.

97. Orlov SN, Koltsova SV, Tremblay J, Baskakov MB, Hamet P. NKCC1 and hypertension: role in the regulation of vascular smooth muscle contractions and myogenic tone. Ann Med. 2012; 44:S111–S118. [PubMed: 22713139]

98. Bianchi G, Ferrari P, Trizio P, et al. Red blood cell abnormalities and spontaneous hypertension in rats. A genetically determined link. Hypertension. 1985; 7:319–325. [PubMed: 3888385]

99. Kotelevtsev, YuV; Orlov, SN.; Pokudin, NI.; Agnaev, VM.; Postnov, YuV. Genetic analysis of inheritance of Na⁺, K⁺ cotransport, calcium level in erythrocytes and blood pressure in F₂ hybrids of spontaneously hypertensive and normotensive rats. Bull Exp Biol Med. 1987; 103:456–458.

100. Meyer JW, Flagella M, Sutliff RL, et al. Decreased blood pressure and vascular smooth muscle tone in mice lacking basolateral Na⁺-K⁺-2Cl⁻ cotransporter. Am J Physiol. 2002; 283:H1846–H1855.

101. Wall SM, Knepper MA, Hassel KA, et al. Hypotension in NKCC1 null mice: role of the kidney. Am J Physiol Ren Physiol. 2006; 290:F409–F416.

102. Lee H-A, Baek I, Seok YM, et al. Promoter hypomethylation upregulates Na⁺-K⁺-2Cl⁻ cotransporter 1 in spontaneously hypertensive rats. Biochem Biophys Res Commun. 2010; 396:252–257. [PubMed: 20406621]

103. Cho H-M, Lee H-A, Kim HY, Han HS, Kim IK. Expression of Na⁺,K⁺-2Cl⁻ cotransporter is epigenetically regulated during postnatal development of hypertension. Am J Hypertens. 2011; 12:1286–1293. [PubMed: 2184290]

104. Mancia G, Grassi G, Giannattasio C, Seravalle G. Sympathetic activation in the pathogenesis of hypertension and progression of organ damage. Hypertension. 1999; 34:724–728. [PubMed: 10523349]

105. Schlaich MP, Lambert E, Kaye DM, et al. Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and angiotensin neuromodulation. Hypertension. 2004; 43:169–175. [PubMed: 14610101]

Genes Dis. Author manuscript; available in PMC 2015 June 23.
106. Huang BS, Amin MS, Leenen FHH. The central role of the brain in salt-sensitive hypertension. Curr Opin Cardiol. 2006; 21:295–394. [PubMed: 16755197]

107. Leenen FHH. The central role of the brain aldosterone- “ouabain” pathway in salt-sensitive hypertension. Biochim Biophys Acta. 2010; 1802:1132–1139. [PubMed: 20230892]

108. Judy WV, Watanabe AM, Henry PD, Besch HR, Murphy WR, Hockel GM. Sympathetic nerve activity: role in regulation of blood pressure in the spontaneously hypertensive rats. Circ Res. 1976; 38:21–29. [PubMed: 178466]

109. Allen AM. Inhibition of the hypothalamic paraventricular nucleus in spontaneous hypertensive rats dramatically reduces sympathetic vasomotor tone. Hypertension. 2002; 39:275–280. [PubMed: 11847197]

110. Li DP, Pan HL. Glutamatergic inputs in the hypothalamic paraventricular nucleus maintain sympathetic vasomotor tone in hypertension. J Pharmacol Exp Ther. 2007; 320:615–626. [PubMed: 17071818]

111. Orlov SN, Resink TJ, Bernhardt J, Buhler FR. Na+–K+ pump and Na+–K+ co-transport in cultured vascular smooth muscle cells from spontaneously hypertensive rats: baseline activity and regulation. J Hypertens. 1992; 10:733–740. [PubMed: 1325504]

112. Gamba G. Role of WNK kinases in regulating tubular salt and potassium transport and in the development of hypertension. Am J Physiol Ren Physiol. 2004; 288:F245–F252.

113. Gamba G. Regulation of NKCC2 activity by SPAK truncated isoforms. Am J Physiol Ren Physiol. 2014; 306:F49–F50.

114. Tang Y, Paney E, Freret T, et al. Effect of hypoxic pre-conditioning on brain genomic response before and following ischemia in the adult mouse: identification of potential neuro-protective candidate for stroke. Neurobiol Dis. 2006; 21:18–28. [PubMed: 16040250]

115. Friso S, Carvajal CA, Fardella CE, Oliveri O. Epigenetics and arterial hypertension: the challenge of emerging evidence. Transl Res. 2015; 165:154–165. [PubMed: 25035152]
127. Orlov SN. NKCC1 as an epigenetically regulated transporter involved in blood pressure elevation with age. Am J Hypertens. 2011; 24:1264. [PubMed: 22083447]

128. Janardhan V, Qureshi AI. Mechanisms of ischemic brain injury. Curr Cardiol Rep. 2004; 6:117–123. [PubMed: 14759356]

129. Folkow B. Cardiovascular “remodeling” in rat anf human: time axis, extent, and in vivo relevance. Physiology. 2010; 25:264–265. [PubMed: 20940430]

130. Liu Y, Gutterman DD. Vascular control in humans: focus on the coronary micocirculation. Basic Res Cardiol. 2009; 104:211–227. [PubMed: 19190954]

131. Bidani A, Griffin KA, Williamson G, Wang X, Loutzenhiser R. Protective importance of the myogenic response in the renal circulation. Hypertension. 2009; 54:393–398. [PubMed: 19546375]

132. Loutzenhiser R, Bidani AK, Wang X. Systolic pressure and the myogenic response of the afferent arteriole. Acta Physiol Scand. 2004; 181:407–413. [PubMed: 15283752]

133. Khodorov B. Clutamate-induced deregulation of calcium homeostasis and mitochondrial dysfunction in mammalian central neurones. Prog Biophys Mol Biol. 2004; 86:279–351. [PubMed: 15288761]

134. Mongin AA. Disruption of ionic and cell volume homeostasis in cerebral ischemia: the perfect storm. Pathophysiology. 2007; 14:183–193. [PubMed: 17961999]

135. Su G, Kintner DB, Sun D. Contribution of Na+,K+,Cl-cotransporter to high-K+o-induced swelling and EAA release is astrocytes. Am J Physiol Cell Physiol. 2002; 282:C1136–C1146. [PubMed: 11940529]

136. Busse S, Breder J, Dinkel K, Reymann KG, Schroder UH. Inhibitors of cation-chloridecotransporters affect hypoxic/hypoglycemic injury in hippocampal slices. Brain Res. 2005; 1946:116–121. [PubMed: 15893737]

137. Su G, Kintner DB, Flagella M, Shull GE, Sun D. Astrocytes from Na+,K+,Cl- cotransporter-null mice exhibit absence of swelling and decrease in EAA release. Am J Physiol Cell Physiol. 2002; 282:C1147–C1160. [PubMed: 11940530]

138. Koltsova SV, Luneva OG, Lavoie JL, et al. HC03-dependent impact of Na+,K+,2Cl- cotransport in vascular smooth muscle excitation-contraction coupling. Cell Physiol Biochem. 2009; 23:407–414. [PubMed: 19471108]

139. Williams RS, Benjamin IJ. Protective responses in the ischemic myocardium. J Clin Invest. 2000; 106:813–818. [PubMed: 11018066]

140. Danielsson J, Yim P, Rinderspacher A, et al. Chloride channel blockage relaxes airway smooth muscle and potentiates relaxation by β-agonists. Am J Physiol Lung Cell Mol Physiol. 2014; 307:L273–L282. [PubMed: 24879056]

141. Haglund MM, Hochman DW. Furosemide and mannitol suppression of epileptic activity in the human brain. J Neurophysiol. 2005; 94:907–918. [PubMed: 15728766]

142. Klonjai W, Lackmy-Vallee A, Katz R, et al. Changes in spinal inhibitory networks induced by furosemide in humans. J Physiol. 2014; 592:2865–2879. [PubMed: 24835171]

143. Hanneart P, Alvarez-Guerra M, Pirot D, Nazaret C, Garay RP. Rat NKCC2/NKCC1 cotransport selectivity for loop diuretic drugs. Schmiedeb Arch Pharmacol. 2002; 365:193–199.

144. Delpire E, Lu J, England R, Dull C, Thorne T. Deafness and imbalance associated with inactivation of the secretory Na-K-2Cl co-transporter. Nat Genet. 1999; 22:192–195. [PubMed: 10369265]

145. Lang F, Vallon V, Knipper M, Wangemann P. Functional significance of channels and transporters expressed in the inner ear and kidney. Am J Physiol Cell Physiol. 2007; 293:C1187–C1208. [PubMed: 17670895]
Figure 1.
The scheme showing implication of cation-chloride cotransporters in the pathogenesis of essential hypertension and its cardiovascular and renal complications. Augmented salt consumption leads to elevation of extracellular fluid volume (EFV) via salt reabsorption by NKCC2 in the thick ascending limb of Henle’s loop (TAHL), NCC in distal tubule (DT) and sensitization of Cl⁻ concentration in tubular fluid delivered to juxtaglomerular apparatus (JGA) by NKCC2. Augmented activity of NKCC1 in vascular smooth muscle cells (VSMC) resulted in elevation of peripheral resistance and systemic blood pressure (SBP). Augmented activity of NKCC1 and attenuated KCC2 in neuronal cells of paraventricular nucleus (PVN) also contributes to SBP elevation via activation of sympathetic nervous system (SNS). On the other hand, augmented NKCC1 activity in VSMC of local microcirculatory bed (LMCB) resulted in suppression of myogenic tone, elevation of local blood pressure (LBP) that, in turn, contributes to the pathogenesis of renal insufficiency, heart failure and stroke.
Table 1

Major characteristics of Cl⁻-coupled cation cotransporters.\(^1,3\)

| Gene    | Human chromosome localization | Protein | Alternative splicing | Tissue localization | Inhibitors, IC\(_{50}\) (μM) |
|---------|-------------------------------|---------|----------------------|---------------------|----------------------------|
| SLC12A2 | 5                             | NKCC1   | NA                   | Ubiquitous          | Bumetanide, 0.05–0.60 Furosemide, 10–50 |
| SLC12A1 | 15                            | NKCC2   | Isoforms A, B and F  | Kidney              | Bumetanide, 0.1–0.5 Furosemide 15–60 |
| SLC12A3 | 16                            | NCC     | NA                   | Kidney              | Polythiazide, 0.5          |
| SLC12A4 | 16                            | KCC1    | NA                   | Ubiquitous          | Bumetanide, 60             |
| SLC12A5 | 20                            | KCC2    | NA                   | Neurones            | Bumetanide, 55 Furosemide 10 |
| SLC12A6 | 15                            | KCC3    | Isoforms A and A     | Neurones            | Bumetanide, 40 Furosemide 25 |
| SLC12A7 | 3                             | KCC4    | NA                   | Ubiquitous          | Bumetanide, 900 Furosemide 900 |

NA, information not available.
Table 2

Effect of ouabain and bumetanide on the volume of human lung fibroblasts.

| Additions                  | Cell volume, pl per cell |
|----------------------------|--------------------------|
| None (control)             | 0.23 ± 0.04              |
| Ouabain                    | 0.26 ± 0.04              |
| Bumetanide                 | 0.22 ±0.01               |
| Ouabain + bumetanide       | 0.43 ± 0.04 *            |

Cells were incubated in the presence of 0.1 μM ouabain and/or 10 μM bumetanide for 24 h. Cell volume was measured as $^{14}$C-urea available space. * Means ± S.E. obtained in experiments performed in quadruplicate are shown.

* $p < 0.005$ compared to control.