NKCC1 as a regulator of vascular tone and a novel target for antihypertensive therapeutics

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The sodium-potassium-chloride cotransporters (NKCCs) belong to the superfamily of Cl⁻-coupled carriers and provide electroneutral symport of Na⁺, K⁺, and Cl⁻. Two NKCC isoforms have been cloned from vertebrate cDNA libraries. NKCC2 is exclusively expressed in the apical membranes of thick ascending limb and macula densa and plays a major role in renal handling of salt and osmotically obliged water. In contrast, NKCC1 is expressed in all types of cells studied so far, including vascular smooth muscle cells (VSMC), and its role in tissue-specific functions remains poorly understood.

Since the 1980s, dozens of researchers have reported that the accelerated turnover of monovalent ions across the plasma membrane of erythrocytes and VSMC, seen in experimental models of primary (essential) hypertension, is at least partially caused by the augmented NKCC activity (14). NKCC was also increased in VSMC from rats with secondary DOCA-salt hypertension (4, 10). Viewed collectively, these data suggest that both genetically determined abnormalities and “hypertensive environments,” such as augmented salt consumption and mineralocorticoid secretion, contribute to the activation of this carrier. Positive correlations between NKCC activity and blood pressure (BP) in F₂ hybrids of spontaneously hypertensive and normotensive rats (14) and attenuated BP in mice lacking NKCC1 (7, 12) strongly indicated the involvement of this carrier in BP regulation.

Under baseline conditions ([Na⁺]o >> [Na⁺]i, and [Cl⁻]o >> [Cl⁻]i, where subscripts i and o represent intracellular and extracellular, respectively), NKCC provides inwardly directed net ion flux and maintenance of intracellular and extracellular, respectively), NKCC provides inwardly directed net ion flux and maintenance of intracellular and extracellular Na⁺, Cl⁻, and K⁺ concentrations and the intracellular pH. Importantly, unlike the dominant contribution of K⁺ permeability (P_K) to resting membrane potential (E_m) in skeletal and cardiac muscles, the ratio of P_K to Cl⁻ permeability in VSMC varies from 0.8 to 0.5 (5). These data suggest that NKCC-mediated modulation of [Cl⁻]i affects E_m and E_m-dependent VSMC contraction. Indeed, high-ceiling diuretics such as furosemide and bumetanide, known to be potent inhibitors of this carrier, decreased [Cl⁻]i (3, 6), hyperpolarized VSMC (6), and attenuated the activation of L-type Ca²⁺ channels (3). These studies disclosed the mechanism underlying the inhibitory action of high-ceiling diuretics documented in an extensive number of studies of vascular and nonvascular smooth muscle contraction (for an updated list of references, see Ref. 16). They also allowed researchers to hypothesize that enhanced NKCC contributes to hypertension via the elevation of vascular tone.

Initial evidence implicating NKCC in the maintenance of vascular tone in vivo was obtained by Meyer and coworkers (12) in experiments on NKCC1 knockout (NKCC1⁻/⁻) mice. In these animals, attenuation of systolic BP by 15–20 mmHg was accompanied by decreased left ventricular pressure without any changes in myocardial contraction parameters. In the paper published in this issue of American Journal of Physiology-Heart and Circulatory Physiology, Garg and coworkers (8) investigated BP in anesthetized rats before and after intravenous bumetanide infusion. They noted that within 10 min of bumetanide infusion, mean arterial BP declined by ~7 mmHg. Clinical studies demonstrated that high-ceiling diuretics decrease BP in patients with volume-expanded hypertension via their accumulation in renal tubular fluid and NKCC2 inhibition. Does NKCC2 contribute to the antihypertensive action of bumetanide observed by Garg and coworkers in anesthetized rats? To answer this question, the authors employed rats in which renal blood flow was blocked by occlusion of both renal arteries. In these animals, the hypertensive action of bumetanide was increased rather than decreased in comparison with the control group. These results demonstrate that the BP-lowering action of bumetanide is not mediated by its interaction with NKCC2.

To prove a key role of NKCC1 in BP regulation, Garg and coworkers (8) measured bumetanide autofluorescence in plasma at excitation and emission wavelengths of 338 and 433 nm, respectively. They revealed that, after bumetanide injection, its concentration in plasma is ~20-fold higher than the IC₅₀ for bumetanide in NKCC1-transfected cells. It should be noted that high plasma levels of nicotinamide adenine dinucleotide and other compounds possessing intensive autofluorescence in the same range as bumetanide complicate usage of the method for precise quantitative analysis. Keeping this in mind, the authors performed experiments with NKCC1 knockout mice. These studies showed that bumetanide infusion did not affect BP in NKCC1⁻/⁻ mice, indicating a key role of NKCC1 in BP regulation.

The final experiments designed by Garg and coworkers (8) examined the role of attenuated tone in resistance arteries under BP declines evoked by bumetanide. They found that contractile responses in phenylephrine-treated isolated third-order mesenteric arteries are accompanied by transient NKCC activation and are attenuated by bumetanide. Importantly, bumetanide sharply reduced hypertension caused by chronic phenylephrine infusion but did not affect the BP elevation evoked by fixed coarctation of the aorta.

Two major conclusions follow from this study. First, NKCC1 is involved in BP adjustment via the regulation of tone in resistance arteries. Second, NKCC1 may be considered a potential pharmacological target in hypertension. Importantly, antihypertensive drugs may exhibit diverse side effects in patients suffering from other diseases. Thus, in asthmatic...
patients, treatment of hypertension with β-blockers is complicated by their actions as bronchoconstrictors. This is not the case with NKCC1 inhibitors that lead to airway smooth muscle relaxation (9).

In conclusion, I would like to formulate several questions that should be answered in forthcoming studies. It is well documented that NKCC activity in cultured VSMC and isolated vessels is activated by vasoconstrictors, such as phenylephrine and angiotensin II, and inhibited by cAMP-raising compounds and other vasodilators (2, 13). Does reciprocal regulation of NKCC1 by vasoconstrictors and vasodilators contribute to vascular tone regulation? In VSMC, NKCC1 is also activated by cell shrinkage and inactivated by cell swelling (15). Does cell volume modulation have an impact on NKCC-mediated VSMC contractile responses?

In erythrocytes with highly active Cl−/HCO3− exchanger, NKCC inhibition does not affect [Cl−]. We have observed that the inhibitory action of bumetanide on VSMC contraction is much higher in HCO3−-free, Tris-buffered medium than in medium buffered with HCO3−-HEPES (3, 11) and employed by Garg and coworkers (8). Additional experiments should be performed to examine the role of HCO3− transporters in the regulation of vascular tone by NKCC1 in vivo.

Endothelium-denuded vessels were used in an overwhelming number of investigations demonstrating the vasodilatory action of NKCC inhibitors. Wang and coworkers (16) reported that treatment with the nitric oxide synthase blocker Nω-nitro-L-arginine methyl ester slowed the dilating action of bumetanide on VSMC and isolated vessels (16). This observation raises questions about the molecular origin of NKCC1-sensitive endothelial elements involved in the regulation of VSMC contraction.

High-ceiling diuretics exhibit about the same efficacy in the inhibition of NKCC1 and NKCC2. At higher concentrations, they also inhibit Na+−-independent K+,Cl− cotransport the activity of which contributes to vasodilatation (1). Side by side with direct actions on VSMC tone, these carriers are involved in [Cl−]-mediated regulation of neuronal activity and may contribute to BP via their impact on sympathetic outflow. All these comments should be considered in the search for new pharmacological tools targeting NKCC1 in hypertension.

REFERENCES


