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## ANO1 taking center stage: Blood pressure regulation in SHR:

### Editorial Comments on “Overexpression of ANO1/TMEM16A, an Arterial Ca<sup>2+</sup>-activated Cl<sup>-</sup> Channel, Contributes to Spontaneous Hypertension

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Calcium-activated chloride channels (CaCCs) were first described in 1981, when injection of Ca<sup>2+</sup> or Ca<sup>2+</sup> ionophore into *Rana pipiens* eggs initiated a transient shift to positive membrane potentials in a chloride-dependent manner [1]. Major biophysical features of CaCCs include: 1) ability of being activated by cytosolic Ca<sup>2+</sup> in the submicromolar range; 2) outward rectification at low intracellular Ca<sup>2+</sup> but display a linear current-voltage relationship at higher intracellular Ca<sup>2+</sup> concentrations; 3) preference to permeate large anions [2]. CaCCs have been implicated in important physiological functions, such as olfactory transduction [3], taste adaptation [4], photosensing [5], fluid secretion [6], synaptic response in neurons [7], cardiac excitability [8], and smooth muscle contraction [9].

For a long period of time, researchers have focused to identify the genes encoding the CaCC channel proteins. However, none of the earlier hits, such as Ca<sup>2+</sup>-activated Cl<sup>-</sup> channel family (CLCA) and Bestrophins, recapitulated properties characteristic of endogenous CaCCs. In 2008, three independent groups successfully decoded TMEM16A (also known as anoctamin 1, ANO1) as a CaCC [9–11]. Schroeder *et al.* cloned *Xenopus* oocyte ANO1 using *Axolotl* oocytes as an expression system, which lacks endogenous CaCC activity [9]. The *Xenopus* oocyte ANO1 expressed from mRNA exhibited characteristic features of calcium and voltage dependence, anion selectivity, and broad expression patterns [9]. Through microarray-based gene expression analysis in IL-4 treated bronchial epithelial cells, Caputo *et al.* showed that ANO1 is associated with calcium-dependent chloride current [10]. By searching public domain databases for putative channel- or transporter-like genes with more than two transmembrane domains and multiple isoforms, Yang *et al.* also pinned down ANO1 as endogenous CaCC [11]. ANO family has ten members in mammals [11]. Besides ANO1, ANO2 has also been confirmed to have endogenous CaCC activity [3]. However, other ANO members do not operate as CaCCs. For example, ANO9 and ANO10 inhibited anion conductance produced by ANO1 [12, 13]. There is evidence that the opening of CaCCs leads to depolarization of the membrane in vascular smooth muscle cells (VSMCs),

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#### Disclosures

The authors declare no conflict of interest.

followed by activation of voltage-dependent  $\text{Ca}^{2+}$  channels (VDCCs), and subsequent contraction [14], which is mediated by ANO1 [15]. This implicates that ANO1 may be involved in the regulation of blood pressure. Indeed, mice lacking ANO1 in vascular smooth muscle have lower systemic blood pressure and a decreased hypertensive response following vasoconstrictor treatment [16].

Spontaneously hypertensive rats (SHRs) have been used as an animal model for human primary or essential hypertension. The SHR strain was established through selective breeding of Wistar Kyoto (WKY) rats with high blood pressure during the 1960s by Okamoto [17]. Therefore, the normotensive WKY rats are always used as controls for SHR in later studies. Because SHRs have a pre-hypertensive state (6–8 weeks, with systolic blood pressures around 100–120 mmHg) [18, 19], they have the potential to be used to reveal mechanistic insights of hypertension development. To date, the precise mechanisms whereby SHRs develop hypertension are still not clear. Its genetic etiology has been much attempted by genome-wide sequencing studies. Although multiple quantitative trait loci (QTL) have been found associated with blood pressure variation, through linkage analyses of crosses between the SHRs and various control strains [20], only Cd36 was identified to represent a specific molecular determinant of hypertension in a model derived from SHR [21]. Recently, meta-analyses of genome-wide association studies (GWASs) have revealed regions of the genome that are significantly associated with blood pressure control in essential hypertension [22]. Johnson *et al.* reported that variants within two genes (rs1801253 in the  $\beta$ -adrenergic receptor and rs11122587 in angiotensinogen) were associated with systolic blood pressure (SBP), diastolic blood pressure (DBP), and hypertension in a study of 86,588 individuals [23]. Another study identified association between systolic or diastolic blood pressure and common variants in eight regions near the *CYP17A1*, *CYP1A2*, *FGF5*, *SH2B3*, *MTHFR*, *c10orf107*, *ZNF652* and *PLCD3* genes [24]. In addition, a larger meta-analysis from 200,000 individuals revealed that 29 independent SNPs were significantly associated with SBP or DBP [25]. Although ANO1 is yet revealed in these studies, data from VSMC specific ANO1 knockout mice strongly suggest a role of ANO1 in blood pressure regulation [16], and that the present study by Wang *et al.* in the current issue of *J. Mol. Cell. Cardiol.* further demonstrated a causal role of ANO1 upregulation in hypertension development in SHRs. It would be interesting to explore whether variants in ANO1 locus are related to hypertension, or whether some genes associated with hypertension are responsible for the regulation of ANO1 expression.

In this issue of the *J. Mol. Cell. Cardiol.*, Wang *et al.* explored the role of ANO1 in the pathogenesis of hypertension in SHRs [26]. The authors found that ANO1 protein abundance was significantly upregulated in both conduit and resistant arteries of SHRs compared to WKYs, and this was accompanied by consistently increased CaCC current. Intriguingly, ANO1 channel inhibitor, T16A<sub>inh</sub>-A01, or siRNA specific for ANO1, lowered blood pressure of SHRs, and inhibited phenylephrine (PE) induced vascular contraction. Ang II treatment of VSMCs increased ANO1 expression via an AT1R/PI3K/Akt signaling pathway. It also increased expression of PCNA in VSMCs, implicating that vascular remodeling in SHRs may be attributed to VSMC targeted overexpression of ANO1. Taken together, these data establish an important role of ANO1 in inducing hypertension in SHRs, likely through enhancing  $\text{Ca}^{2+}$  influx and VSMC proliferation.

ANO1 has been described to express in a broad range of tissues, including a variety of epithelia, sensory cells and smooth muscles [27]. One of the novel findings in this present study is the upregulation of ANO1 protein in a series of small and large arteries in SHR. The authors defined a signaling pathway of AT1R/PI3K/Akt in VSMCs that is responsible for Ang II dependent upregulation of ANO1. It would be of interest to see if endogenously produced Ang II or activation of Ang II pathway in SHR would activate the same response. It would also be interesting to translate these novel findings into future clinic application. Inhibitors of Ang II pathway such as ACEI and AT1RB, as well as blockers of VDCC that lies downstream of CaCC, have been used in the clinic to treat hypertension. In order to reduce side effects of these drugs, or improve the therapeutic efficacy, there have been successful cases of combinatory therapy. For example, combination of telmisartan (AT1R blocker) and lercanidipine (calcium channel blocker) at lower doses are effective in lowering BP, and reducing side effects caused by maximal doses of each drug [28]. Identification of ANO1/VDCC signaling as an important component in blood pressure regulation in the SHR in particular, may facilitate development of combinatory therapies more effective in attenuating this pathway in essential hypertension.

Recently, Mazzone *et al.* showed that IL-4 induces ANO1 expression in a STAT6 (signal transducer and activator of transcription 6)-dependent manner [27]. In addition, others have shown a role of ANO1 upregulation in tumorigenesis in some cancers, although the mechanisms are not clear [29, 30]. Whereas understanding mechanisms of ANO1 upregulation may prove to be beneficial in designing novel anti-hypertensive drugs, another group found downregulation of ANO1 in the hypertensive basilar artery, and that Ang II inhibited ANO1 expression in basilar smooth muscle cell [31]. These data may indicate differential regulations of ANO1 in different types of hypertension and vascular remodeling.

Clearly, CaCC is a unique type of chloride channel that is regulated by  $Ca^{2+}$ , and is involved in several important physiological processes. The current study by Wang et al provides convincing evidence that upregulation of the CaCC ANO1, plays critical role in the development of hypertension in SHR. This finding may courage more investigation of ANO1 in the setting of human essential hypertension. Identification of potential relationships between ANO1 variants and hypertension, revelation of mechanisms underlying ANO1 upregulation, and development of new anti-hypertensive drugs based on the ANO1 pathway, may serve as worthy future directions to explore.

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