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Original article

Transgenic overexpression of translationally controlled tumor protein induces systemic hypertension via repression of Na^+,K^+-ATPase

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Abstract

Inhibition of Na^+,K^+-ATPase has been implicated in the pathogenesis of hypertension via its effect on smooth muscle reactivity and myocardial contractility. We recently demonstrated that translationally controlled tumor protein (TCTP) interacts with the 3rd cytoplasmic domain of Na^+,K^+-ATPase α_1-subunit and acts as its cytoplasmic repressor. Therefore, we hypothesized that repression of Na^+,K^+-ATPase by overexpressed TCTP might underlie the development of hypertension. In the present study, we confirmed that transgenic mice overexpressing TCTP developed systemic arterial hypertension at about 6 weeks after birth. Vascular smooth muscle of TCTP-overexpressing transgenic mice also displayed augmented contractile response to vasoconstrictors and attenuated relaxation response to vasodilators. These responses seem to be caused by reduced Na^+,K^+-ATPase activity and increased intracellular calcium, suggesting that inhibition of Na^+,K^+-ATPase by overexpression of TCTP is involved in the pathogenesis of hypertension. This study provides a new link between alteration of sodium pump activity and hypertension *in vivo*, and suggests that TCTP might be a therapeutic target for the treatment of hypertension.

Keywords: Na^+; K^+-ATPase; Translationally controlled tumor protein (TCTP); Hypertension; Vascular contractility; Calcium

1. Introduction

Systemic hypertension is a complex and common disorder, and is a risk factor for other cardiovascular diseases, such as stroke and renal failure [1,2]. The pathogenesis of hypertension remains incompletely understood.

Na^+,K^+-ATPase present in all eukaryotic cell membranes influences blood pressure by controlling cellular ion homeostasis and membrane potential [3], thereby contributing to the regulation of smooth muscle and myocardial contractility and tone in the vasculature [4–7]. Long-term administration of ouabain, an Na^+,K^+-ATPase inhibitor, produced hypertension in rats [8,9], and high levels of endogenous ouabain-like compounds are implicated in the development and/or maintenance of hypertension [10–12]. Many forms of hypertension are associated with intracellular Ca^{2+} augmentation through Na/Ca-exchanger (NCX), membrane depolarization, and norepinephrine release from perivascular nerve endings, all of which promote vasoconstriction [4,5,8].

TCTP is a growth-related protein under both transcriptional and translational controls [13]. TCTP is found in all cell types and is highly conserved among various species. The primary sequence of TCTP shows no homology to any other protein family, but exhibits some similarity to the small Mss4/Dss4 chaperone family [14]. TCTP levels have been reported to correlate with cell cycle progression [15] and malignant transformation [16]. TCTP

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appears to have multiple functions including anti-apoptotic activity [17] and histamine-releasing activity, an ability to trigger histamine secretion from basophils [18]. However, its primary physiological function remains unknown.

It has been suggested that dysregulation of Na^+,K^+-ATPase activity by other proteins and hormones [19] might underlie disease states such as hypertension. In our earlier studies we have shown that TCTP acts as a cytoplasmic repressor of Na^+, K^+-ATPase in HeLa cells by interacting with the 3rd cytoplasmic domain of its catalytic α-subunit [20]. We report here that overexpression of TCTP in vivo leads to hypertension through the inhibition of vascular Na^+,K^+-ATPase activity and subsequent intracellular calcium mobilization and smooth muscle vasoconstriction.

2. Methods

2.1. Transgenic mice

Transgenic (TG) mice overexpressing TCTP were generated in C57BL/6+CBA hybrid background using the targeting construct pCAGGS-TCTP cDNA containing CMV-IE and chicken β-actin promoter as described [21], and backcrossed with C57BL/6 mice (Online supplement). Over-expression of TCTP was confirmed by PCR, Southern blotting, Northern blotting, and finally Western blotting (Supplementary Fig. 1). All animal experiments were performed according to the National Institutes of Health Publication No. 8523: Guide for the Care and Use of Laboratory Animals.

2.2. Hemodynamic measurements

Following echocardiography (Online supplement), hemodynamic evaluation was performed in mice. A 1.4-F high-fidelity micromanometer catheter (Model SPR-671, Millar Instruments, USA) was inserted into the right carotid artery and advanced retrograde into the left ventricle [LV] [22]. Heart rate, arterial pressure, LV-systolic pressure (LVSP), and LV-end diastolic pressure (LVEDP) were recorded.

2.3. Isometric force measurement on isolated aortic rings

Isometric force was measured on denuded thoracic aortic rings isolated from 10- to 12-week-old NTG and TG mice, as described [23]. Absence of endothelium was confirmed by the absence of response to 3×10^{-6} mol/L acetylcholine. Norepinephrine (NE), serotonin (5-HT), high K^+ and sodium nitroprusside (SNP) were used to test vasomotion.

2.4. Vascular smooth muscle cell culture

Vascular smooth muscle cells (VSMCs) were isolated from thoracic aortas of 10- to 12-week-old NTG and TG mice, with modifications [24]. After digestion of the aorta with collagenase type II (175 units/mL, Worthington) at 37 °C for 10 min, adventitia and endothelium were removed, and the stripped aorta was chopped and further digested with a mixture of collagenase type II (175 units/mL) and elastase (0.2 mg/mL; Sigma) for 50–80 min at 37 °C. Cells were grown in DMEM containing 10% FBS and antibiotics. SMC lineage was confirmed by the presence of immunoreactivity to smooth muscle α-actin (Sigma). Cells at passage number 3–6 were used in these studies.

2.5. $^{86}Rb^+$ uptake assay [20]

The VSMCs were incubated with or without 2.5 mmol/L (inhibits the total Na^+,K^+-ATPase activity ($\alpha_1+\alpha_2$)) or 1 μmol/L (inhibits only α_2 activity) ouabain and/or 1 mmol/L furosemide for 15 min at 37 °C, and for an additional 15 min after addition of ^{86}RbCl (1.2 μCi/mL). The total Na^+,K^+-ATPase activity ($\alpha_1+\alpha_2$) was determined by subtracting 2.5 mmol/L ouabain-resistant $^{86}Rb^+$ uptake from the total uptake, and α_2 activity, by subtracting [1 μmol/L ouabain+furosemide]-resistant uptake from furosemide-resistant uptake as described by Coppi and Guidotti [25]. The furosemide-sensitive uptake corresponds to Na,K, Cl-cotransporter (NKCC) activity.

2.6. Resting $[Ca^{2+}]_{cyt}$ and $[Na^+]_{cyt}$ measurements

Resting $[Ca^{2+}]_{cyt}$ and $[Na^+]_{cyt}$ in 10^4 quiescent VSMCs were determined by flow cytometry with a FACS (Becton-Dickinson, MA, USA) using the Ca^{2+}-sensitive indicator, fluo-3 AM and the Na^+ indicator, Sodium Green AM (Molecular Probes, OR, USA), respectively [26,27]. HEPES-buffered balanced salt solutions used in these studies contained (in mM): NaCl 126, KCl 4.4, $CaCl_2$ 1.08, $MgCl_2$ 1.0, HEPES 24, glucose 5 and probenecid 0.5 (pH 7.4). Propidium iodide 25 μmol/L (Molecular Probes) was added 30–50 s prior to data acquisition as a marker for nonviability.

2.7. Confocal microscopy

Changes of $[Ca^{2+}]_{cyt}$ of VSMCs in supplemented DMEM medium were monitored by laser scanning confocal microscopy (Zeiss LSM 410) as previously described [28]. 5-HT, which mobilizes Ca^{2+} from the endoplasmic reticulum (ER), was added to the cells with or without pre-treatment of 1 mmol/L ouabain or 5 mmol/L EGTA for 60 s [29,30]. The changes in $[Ca^{2+}]_{cyt}$ were represented by relative fluorescence intensity (F/F_0, RFI).

2.8. Statistical analysis

Data are presented as mean±SEM. Between-group comparisons were analyzed by the nonparametric Mann–Whitney test. Significance was assumed at $P<0.05$.

3. Results

3.1. High blood pressure induced in TCTP-overexpressing transgenic mice

We generated three independent TG mice lines and confirmed overexpression of TCTP (Supplementary Fig. 1). Two