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Calcium-dependent expression of transient receptor potential canonical type 3 channels in patients with chronic kidney disease

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ABSTRACT

It is unknown whether extracellular calcium may regulate the expression of transient receptor potential canonical type 3 (TRPC3) channels in patients with chronic kidney disease.

Using quantitative in-cell Western assay we compared the expression of TRPC3 channel protein in monocytes from 20 patients with chronic kidney disease and 19 age- and sex-matched healthy control subjects. TRPC3 channels were identified by immunoblotting using specific antibodies and TRPC3 protein was further confirmed by mass spectrometry.

We observed a significant increase of TRPC3 channel protein expression in patients with chronic kidney disease compared to healthy control subjects (normalized expression, 0.42 ± 0.06 vs. 0.19 ± 0.03; $p < 0.01$). Expression of TRPC3 was significantly inversely correlated with estimated glomerular filtration rates (Spearman $r = -0.41$) or serum calcium concentration (Spearman $r = -0.34$). During a hemodialysis session serum calcium concentrations significantly increased, whereas the expression of TRPC3 channels and calcium influx significantly decreased. In vitro studies confirmed that higher calcium concentrations but not magnesium, barium nor sodium concentrations significantly decreased TRPC3 expression in human monocytes.

This study indicates that reduced extracellular calcium concentrations up-regulate TRPC3 channel protein expression in patients with chronic kidney disease.

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Introduction

Disturbances of extracellular calcium concentrations are well-known in patients with chronic kidney disease (CKD)[1]. In patients with CKD alterations in vitamin D metabolism, decreased levels of calcitriol, and decreased serum calcium concentrations are commonly observed [1]. Changes of calcium homeostasis and calcium transport systems due to decreased serum calcium concentrations may be involved in the pathogenesis of hypertension, arteriosclerosis, vascular calcifications and cardiovascular disease which is frequently observed in uremia [2–4]. From recent literature there is evidence that changes of extracellular calcium can directly affect cytosolic free calcium homeostasis and calcium transport systems in human monocytes [4]. Cytosolic free calcium concentration is tightly regulated. Mechanisms for increasing cytosolic calcium include the release of calcium from intracellular stores and calcium influx across the plasma membrane [5]. Calcium influx can be mediated by non-selective cation channels, including members of the transient receptor potential canonical (TRPC) channel family [6]. TRP channel-mediated calcium influx has been identified in several cell types, including human peripheral blood cells [7–9]. Recent experimental results were published showing that the activation of the calcium-sensing receptor increases TRPC3 expression in rat cardiomyocytes [10]. To the best of our knowledge there are no reports how extracellular calcium may regulate TRPC3 expression in humans. Furthermore, the regulation of TRPC3 channels in patients with CKD has not been investigated yet. The purpose of the present study was to investigate whether extracellular calcium may regulate the expression of TRPC3 channels in patients with CKD. Our present study demonstrates that reduced extracellular calcium concentration up-regulates TRPC3 channel protein expression in patients with CKD.

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[1] *Abbreviations used:* CKD, chronic kidney disease; TRPC, transient receptor potential canonical; eGFR, estimated glomerular filtration rate; HEPES, *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid; MALDI-TOF, matrix assisted laser desorption/ionisation time of flight.

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Materials and methods

Subjects

We studied 20 patients with CKD (16 men, 4 women) and 19 healthy control subjects (13 men, 6 women). CKD was defined by kidney damage as confirmed by kidney biopsy or markers of damage, or estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 m^2 for more than 3 months. Markers of kidney damage included proteinuria, abnormalities on the urine dipstick or sediment examination, or abnormalities on imaging studies of the kidneys. Patients with an age less than 18 years, pregnancy, or refusal of giving informed consent were excluded. Blood pressure was measured 3 times with a sphygmomanometer after 10 min of recumbency and averaged. Renal function was quantified by eGFR which was calculated according to the Modification of Diet in Renal Disease formula [11]. Of the patients with CKD, 8 patients (40%) had diabetes mellitus, 7 patients (35%) had a history of coronary artery disease, 5 patients (25%) had peripheral vascular disease and 2 patients (10%) had cerebrovascular disease. Seven patients (35%) were smokers. Additional measurements were performed in monocytes which were obtained from patients with CKD stage 5 immediately at the start and at the end of a hemodialysis session. These patients were routinely dialyzed for 4 h 3 times weekly for more than 3 months using biocompatible membranes. The dialysates used were bicarbonate-based. The standard dialysate contained 1.75 mmol/L calcium. In these hemodialysis patients K_t/V values (the amount of plasma cleared of urea divided by the urea distribution volume) were higher than 1.2. No patient had significant pain, elevated temperature or other acute conditions. Healthy control subjects were recruited among patients with minor complaints. Subjects with major medical illnesses were excluded. All subjects gave written informed consent and the study was approved by the local ethics committee.

Preparation of cells

Human monocytes were obtained from heparinized blood using superparamagnetic polystyrene beads coated with a primary monoclonal antibody specific for the CD14 membrane antigen expressed on human monocytes (Invitrogen, Germany) and resuspended in Hanks balanced salt solution containing (in mmol/L) NaCl 136, KCl 5.40, KH_2PO_4 0.44, Na_2HPO_4 0.34, D-glucose 5.6, $CaCl_2$ 1, *N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid (HEPES) 10, pH 7.4. To evaluate the effects of extracellular cations on TRPC channel protein expression experiments were performed in which human monocytes were incubated in solutions containing different concentrations of calcium, magnesium, sodium, or barium for 4 h prior to measurements of TRPC expression. An incubation time of 4 h in vitro was chosen to mimic the duration of a hemodialysis session.

Immunoblotting of TRPC channels from human monocytes

Monocytes were washed with phosphate-buffered saline, transferred to ice-cold high-salt lysis buffer containing (in mmol/L) Tris–HCl 25, pH 8.0; NaCl 100, ethylenediaminetetraacetic acid 2, β-mercaptoethanol 20, sodium fluoride 50, complete mini protease inhibitor cocktail (Roche Diagnostics), repeatedly aspirated through a syringe needle. Proteins were separated by 10% sodium-dodecylsulfate–polyacrylamide-gelelectrophoresis (SDS–PAGE) at 150 V for 90 min, and transferred to Hybond-ECL nitrocellulose membranes (NEN Life Science Products, Boston, MA). Membranes were blocked with Odyssey blocking buffer (Licor biosciences, Bad Homburg, Germany) for 24 h at 4 °C. Membranes were incubated with commercially available rabbit anti-human TRPC3 antibodies (Alomone labs, Jerusalem, Israel) as described by several investigators [8,12,13] at 1:500 solution containing 0.1% Tween 20 for 60 min. Membranes were washed and were then incubated with IR-Dye800CW-infrared fluorescent dye-conjugated sheep anti-rabbit antibody (1:1000, Biomol, Hamburg, Germany) and Alexa Fluor680-allophyco cyanin-fluorescence-labeled donkey anti-goat antibodies (1:1000, Invitrogen) for 60 min. Imaging was performed using the Odyssey infrared imaging system (Licor Biosciences, Bad Homburg, Germany) at 810 nm emission with an excitation wavelength of 780 nm, and at 700 nm emission with an excitation wavelength of 680 nm. These experiments confirmed the molecular mass of the protein and showed that the antibodies can be used to identify TRPC3 channels in human monocytes.

Identification of TRPC using matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry

The isolated TRPC3 was identified using MALDI-TOF/TOF mass-spectrometry. The gel plug of the gel-electrophoresis was washed and equilibrated with aqueous ammonium bicarbonate (50 mmol/L) and acetonitrile (50:50 vol.%) and digested with aqueous ammonium bicarbonate (50 mmol/L) and 0.5% w/v trypsin (37 °C; 24 h). The peptides were desalted utilizing the PerfectPure C-18ZipTip (Eppendorf, Hamburg, Germany) technology, and eluted directly with 0.3% trifluoracetic acid (TFA) and 60% acetonitrile in water onto the MALDI target plate (MTP AnchorChip 400/384; Bruker-Daltronic, Bremen, Germany) using a-cyano-4-hydroxycinnamic acid (2.5 mg/mL) as matrix. The subsequent mass-spectrometric analyses were carried out using a MALDI-TOF/TOF-spectrometer (Ultraflex III; Bruker-Daltonic, Bremen, Germany). The annotated spectra were subjected to a database search (Swiss-Prot, Zurich, Switzerland) utilizing Bruker-Daltonic BioTools (vers. 3.1) and the Mascot search engine (vers. 2.2), which compares the experimental MALDI-MS data set with the calculated peptide masses for each entry in the sequence database (http://www.matrixscience.com).

Quantitative in-cell Western assay for TRPC channel protein expression

For quantitative measurements of TRPC channel proteins in-cell Western assays of human monocytes were performed using the

Table 1
Clinical and biochemical characteristics of healthy control subjects and patients with chronic kidney disease (CKD).

Characteristic	Healthy control subjects	Patients with CKD
Age (years)	63 ± 3	64 ± 3
N (male/female)	13/6	16/4
Systolic blood pressure (mmHg)	120 ± 1	129 ± 6
Diastolic blood pressure (mmHg)	69 ± 2	69 ± 4
Pulse pressure (mmHg)	51 ± 2	60 ± 3
Heart rate (per minute)	78 ± 3	79 ± 4
Body mass index (kg/m^2)	26.5 ± 0.8	27.8 ± 1.4
Hemoglobin (g/L)	131 ± 3	114 ± 4*
Leukocytes (G/L)	9.0 ± 1.0	10.5 ± 0.9
Platelets (G/L)	222 ± 16	269 ± 29
Serum sodium (mmol/L)	136 ± 1	137 ± 1
Serum potassium (mmol/L)	3.9 ± 0.1	4.2 ± 0.2
Serum calcium (mmol/L)	2.25 ± 0.02	2.16 ± 0.03*
Serum phosphate (mmol/L)	0.84 ± 0.05	1.48 ± 0.12*
Cholesterol (mg/dL)	168 ± 12	202 ± 21
Glucose (mg/dL)	114 ± 6	132 ± 11
Creatinine (mg/dL)	0.8 ± 0.0	3.7 ± 0.6*
Estimated glomerular filtration rate (mL/Min/1.73 m^2)	84 ± 4	27 ± 4*

Data are mean ± SEM.
* $p < 0.05$ compared to healthy control subjects.

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