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DISSERTATION

Spannungsabhängige $Ca_V3.2$-Kanäle und Ca^{2+}-Sparks in der glatten Gefäßmuskulatur der Mesenterialarterie
Voltage-gated $Ca_V3.2$ channels and Ca^{2+} sparks in mesenteric artery vascular smooth muscle

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Abstract

Background: Previousresearchessuggest that T-type $Ca_v3.2$ channels in arterial vascular smooth muscle cells (VSMCs) and caveolae have contribution on elementary Ca^{2+} signaling (Ca^{2+} sparks) *via* ryanodine receptors (RyRs) to cause vasodilation. RyRs are major Ca^{2+}-release channels in the sarcoplasmic reticulum membrane of myocytes.VSMCs exhibit 3 differentisoforms of RyR (RyR1, RyR2, and RyR3).Here, we aim to elucidate the contribution of caveolar $Ca_v3.2$ channels and their functional interaction with $Ca_v1.2$ channels to trigger Ca^{2+} sparks in VSMCs. We also tested the hypothesis that vascular smooth muscle cell RyR2 play a specificrole in Ca^{2+}sparks generation in VSMCs.

Methods: We used tamoxifen-inducible smooth muscle-specific $Ca_v1.2^{-/-}$ (SMAKO) mice, tamoxifen-inducibleSmooth muscle cell–specific $RyR2^{-/-}$ mice and laser scanning confocal microscopy to assess Ca^{2+} sparks in arterial VSMCs. Ni^{2+}, Cd^{2+} and methyl-ß-cyclodextrin were used to inhibit $Ca_v3.2$ channels, $Ca_v1.2$ channels, and caveolae, respectively.

Results: We found that 50 µmol/L Ni^{2+} and 10 mmol/Lmethyl-ß-cyclodextrindecreased Ca^{2+} spark frequency by ~20-30% in mesenteric artery VSMCs in a non-additive manner,but failed to inhibit Ca^{2+} sparks in tibial and cerebral artery VSMCs. 200 µmol/L Cd^{2+} suppressed Ca^{2+} sparks in mesenteric arteries by ~70-80%. A similar suppression of Ca^{2+} sparks was seen in mesenteric artery VSMCs of SMAKO mice. The remaining Ca^{2+} sparks were fully abolished by Ni^{2+} or methyl-ß-cyclodextrin. Our results demonstrate that Ca^{2+} influx through $Ca_V1.2$ channelsis the primary means of triggeringCa^{2+} sparks in murine arterial VSMCs. $Ca_V3.2$ channels, localized to caveolae and tightly coupled to RyR, provide an additional Ca^{2+} source for Ca^{2+} spark generation in mesenteric, but not tibial and cerebral arteries. In addition, targeted deletion of the RyR2 gene resulted in a complete loss of sarcoplasmic reticulum–mediated Ca^{2+}-release events.

Conclusions: Here we demonstrate that L-type $Ca_V1.2$ channels provide the predominant Ca^{2+} pathway for Ca^{2+} sparksgeneration in murine arterial VSMCs. T-type $Ca_v3.2$ channels are located in pits structures of caveolae to provide locally restricted, tight coupling between T-type Ca^{2+} channels and RyRs to ignite Ca^{2+} sparks in mesenteric arteries.Furthermore, RyR2 isoform plays a dominant role in local and global VSMCs SRCa^{2+} release.

Zusammenfassung

Hintergrund: Bisherige Untersuchungen ließen vermuten, dass T-Typ $Ca_V3.2$-Kanäle in arteriellenGefäßmuskelzellen (VSMCs) und Caveolae an elementaren Ca^{2+}-Signalprozessen (Ca^{2+}-Sparks) über Ryanodinrezeptoren (RyRs)zum Auslösen von Vasodilatation beteiligt sind. RyRs sind wichtige Ca^{2+}-Freisetzungskanäle in der Membran des Sarkoplasmatischen Retikulums (SR) von Myozyten. VSMCs exprimieren 3 verschiedene Isoformen von RyR (RyR1, RyR2 und RyR3). Das Ziel dieser Studie war, den Beitrag der caveolaren $Ca_V3.2$-Kanäle und deren funktionelle Interaktion mit $Ca_V1.2$-Kanäle beim Auslösen von Ca^{2+}-Sparks in VSMCs aufzuklären. Wir überprüften weiterhin die Hypothese, dass RyR2 in Zellen der glatten Gefäßmuskulatur eine spezifische Rolle bei der Erzeugung von Ca^{2+}-Sparks spielen.

Methoden: Wir nutzten Tamoxifen-induzierbare Glattmuskel-spezifische $Cav1.2^{-/-}$ (SMAKO) Mäuse, Tamoxifen-induzierbare Glattmuskel-spezifische $RyR2^{-/-}$-Mäuse und Laser-Scanning-Konfokalmikroskopie,um Ca^{2+}-Sparks in VSMCs zu untersuchen. Ni^{2+}, Cd^{2+}und Methyl-ß-cyclodextrin wurden verwendet, um $Ca_V3.2$-Kanäle oder $Ca_V1.2$-Kanäle zu hemmenbzw. Caveolae aufzufalten.

Ergebnisse: Wir haben festgestellt, dass 50 µmol/L Ni^{2+} und 10 mmol/L Methyl-ß-cyclodextrin die Frequenz der Ca^{2+}-Sparks um ~20-30% in VSMCs aus Mesenterialarterien nicht-additiv verminderten, aber Ca^{2+}-Sparks bei VSMCs aus Tibial- und Zerebralarterien nicht hemmen konnten. 200 µmol/L Cd^{2+}unterdrückte ~70–80% der Ca^{2+}-Sparks in VSMCs aus Mesenterialarterien. Eine ähnliche Hemmung von Ca^{2+}-Sparks fanden wir in VSMCs aus Mesenterialarterien von SMAKO-Mäusen. Die verbliebenen Ca^{2+}-Sparks wurden durch Ni^{2+} oder Methyl-ß-cyclodextrin vollständig gehemmt. Unsere Ergebnisse zeigen, dass Ca^{2+}-Einstrom durch $Ca_V1.2$-Kanäle der Hauptweg zum Auslösen von Ca^{2+}-Sparks in murinen arteriellen VSMCs ist. $Ca_V3.2$-Kanäle, lokalisiert in Caveolae und dicht gekoppelt an RyRs, bieten eine zusätzliche Ca^{2+}-Einstromquelle zur Erzeugung von Ca^{2+}-Sparks bei Mesenterial-, aber nicht bei Tibial- oder Zerebralarterien. Darüber hinaus führte die gezielte Deletion des RyR2-Genszueinemvollständigen Verlust von SR-vermitteltenCa^{2+}-Freisetzungsereignissen.

Schlussfolgerungen: Wir konnten zeigen, dass L-Typ $Ca_V1.2$-Kanäle den Haupt-Ca^{2+}-Einstromweg zur Bildung von Ca^{2+}-Sparks in murinen arteriellen VSMCs darstellen. T-Typ $Ca_V3.2$-Kanäle sind innerhalb von Caveolae lokalisiert, um lokal begrenztes, enges Koppeln zwischen den $Ca_V3.2$-Kanälen und RyRs zum Auslösen von Ca^{2+}-Sparks in mesenterialarteriellen VSMCs zu ermöglichen. Außerdem spielt die RyR2-Isoform eine dominante Rolle bei der lokalen und globalen Freisetzung von VSMCs SR-Ca^{2+}.

1. Introduction

In resistance arteries, voltage dependent Ca^{2+} channels activate ryanodine receptors (RyRs) to cause elementary Ca^{2+} release events (Ca^{2+} sparks) from the sarcoplasmic reticulum (SR). Ca^{2+} release from the SR in the form of Ca^{2+} sparks opens numerous large-conductance Ca^{2+}-sensitive K^+ (BK_{Ca}) channels, causing spontaneous transient outward K^+ currents (STOCs) [1, 2]. As a result, Ca^{2+} spark–BK_{Ca} channel coupling induces vascular smooth muscle cell (VSMCs) hyperpolarization and attenuation of arterial constriction [3-5].

Voltage-depended L-type $Ca_V1.2$ channels provide an important pathway for influx of extracellular Ca^{2+} and their graded activation plays a central role in setting cytosolic [Ca^{2+}] in smooth muscle contraction. Blocking $Ca_V1.2$ pharmacologically or using $Ca_V1.2$ specific knockout mice causes a reduction in the frequency of Ca^{2+} spark events and in myogenic tone [6].Local or tight coupling of the $Ca_V1.2$-RyRs isn't necessary to Ca^{2+} sparks generation. L-type $Ca_V1.2$ channels influenced Ca^{2+} spark through their ability to set global cytosolic [Ca^{2+}] and consequently the SR refilling rate. Intriguingly, Ca^{2+} sparks were not completely eliminated following $Ca_V1.2$ channel ablation, a finding which suggests the presence of an additional Ca^{2+} influx pathway in Ca^{2+} spark generation. Recent studies show that voltage dependent T-type channels ($Ca_V3.2$) could induce RyR mediated Ca^{2+} sparks [7,8.] In this study, Ni^{2+}, a selective $Ca_V3.2$ channel blocker, suppressed Ca^{2+} sparks and consequently STOCs[7]. Transmission electron microscopy and immunogold labeling revealed that $Ca_V3.2$ channels are located in or close to caveolae, pit structures in the plasma membrane, which have been implicated earlier in Ca^{2+} spark ignition[8]. Nevertheless, it remains largely unknown whether T-type $Ca_V3.2$ channels are functional in caveolae, and how they contribute to elementary Ca^{2+} signaling in VSMCs.

RyRs are major Ca^{2+}-release channels in the SR membrane of myocytes that contribute to the regulation of contractility.VSMCs exhibit 3 different RyR isoforms (RyR1, RyR2, and RyR3), while the contributions of the RyR2 subtype to arterial elementary Ca^{2+} signaling in the vasculature remain unclear. Therefore, we generated RyR2-deficient mice and tested the hypothesis that vascular smooth muscle cell RyR2s play a specificrole in elementary Ca^{2+} signaling.

2. Aims and Hypotheses

The main purposes of the experimental work for my thesis were to study the roles of T-type $Ca_V3.2$ channels in RyRs-BK_{Ca} signaling in mesenteric artery vascular smooth muscle cells and their functional interaction withRyR2isoforms and ultrastructural requirements in the plasma cell membrane:

Hypothesis #1:

We tested the hypothesis that T-type $Ca_V3.2$ channels play a role in triggering Ca^{2+} sparks in VSMCs.These channels are located in pits structures of caveolae to provide locally restricted, tight coupling between T-type $Ca_V3.2$ channels and RyRs to ignite Ca^{2+} sparks.

Hypothesis #2:

We tested the hypothesis that L-type $Ca_V1.2$ channels provide the predominant Ca^{2+} pathway for the generation of Ca^{2+}sparks in murine arterial VSMCs, withT-type $Ca_V3.2$ channels representing an additional source for generation of the Ca^{2+} sparks.

Hypothesis #3:

We tested the hypothesis that vascular smooth muscle cell RyR2 play a specificrole in elementary Ca^{2+} signaling.

3. Methods

3.1 Animal preparation.

At an age of 2-3 months, male mice deficient in the smooth muscle $Ca_V1.2$ Ca^{2+} channel (SMAKO, smooth muscle α1c-subunit Ca^{2+} channel knockout),malemice deficient in the smooth muscle Ryr2(Ryr2flox/flox;SM22a-CreT2 orRyr2flox/flox;SM22a-CreT2/T2) and corresponding control mice ($Ca_V1.2^{+/+}$; SM22α-$Cre^{T2/T2}$, $Ca_V1.2^{+/+}$; SM22α-Cre^{T2}, $Ca_V1.2^{+/+}$, $Ca_V1.2^{flox/+}$ or $Ca_V1.2^{flox/flox}$) (Ryr2+/+;SM22a-CreT2/T2,Ryr2+/+;SM22a-CreT2,Ryr2+/+,Ryr2flox/+, or Ryr2flox/flox)were i.p. injected with tamoxifen (30 µg/g body weight day^{-1}) for five consecutive days. After 2-4 days, mice were deeply anaesthetized by inhalation of isoflurane until cessation of breathing, thenkilled by cervical dislocation and the brain, mesentery and tibial arteries removed. Experiments were performed on the same day with arteries from litter-matched control and SMAKO mice.

3.2 Isolation of arterial vascular smooth muscle cells.

Arteries were removed and quickly transferred to cold (4°C) oxygenated (95% O_2-5% CO_2) physiological salt solution (PSS). The arteries were cleaned, cut into pieces, and placed into a Ca^{2+}-free Hank‘s solution (mM) for 37 min at 37°C. The segments were then placed in Hank‘s solution containing 1 mg/ml collagenase (Sigma, type F and H, ratio 30% and 70%) and 0.1 mM $CaCl_2$ for 17 min at 37°C. Following several washes in Ca^{2+}-free Hank‘s solution, single cells were dispersed from artery segments by gentle triturating. Cells were then stored in the same solution at 4°C. In some experiments, Ca^{2+} imaging was performed on isolated mesenteric artery segments. The arteries were cleaned, cut into pieces, and placed into a Ca^{2+}-free Hank‘s solution containing 0.5 mg/ml papain (Sigma) and 1.0 mg/ml DTT for 37 min at 37°C. Arterial segments were then stored in the same solution at 4°C.

3.3 Calcium imaging measurements.

Isolated VSMCs or artery segments were seeded onto glass coverslips and incubated with the Ca^{2+} indicators fluo-4 AM (10 µmol/L) and pluronic acid (0.005%, w/v) for 60 min at room temperature in Ca^{2+}-free Hanks’ solution.After loading the cells were washed with bath solution for at least 10 min at room temperature. VSMCs were imaged (at 20 or higher (up to 59) frames/s) using a Nipkow disc-based UltraView LCI confocal linked to a fast digital camera. The confocal system was mounted in an inverted Diaphot microscope with a x40 oil-immersion objective (NA 1.3, Nikon). Images were obtained by illumination with an argon laser at 488 nm, and recording all emitted light above 515 nm. Ca^{2+} spark analysis was performed off-line using

the Ultra View Imaging Suite software (Perkin Elmer). Ca^{2+} sparks were defined as local fractional fluorescence increase clearly above the noise level. The frequency was calculated as the number of detected sparks divided by the total scan time.

3.4 Electrophysiology.

Potassium currents were measured in the whole-cell perforated-patch mode of the patch-clamp technique.Patch pipettes were filled with a solution containing (in mM): 110 K-Asp, 30 KCl, 10 NaCl, 1 $MgCl_2$, and 0.05 EGTA (pH 7.2). The patch pipette solution was supplemented with 200 µg/ml Amphotericin B, dissolved in dimethyl sulfoxide (DMSO), to measure K^+ currents in the whole-cell perforated-patch mode. The external bath solution contained (in mM): 134 NaCl, 6 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose and 10 HEPES (pH 7.4); holding potential was -60 mV. Whole cell currents were recorded using an Axopatch 200B amplifier or an EPC 7 amplifier at room temperature. Data were digitized at 5 kHz, using a Digidata 1440A digitizer and pClamp software versions 10.1 and 10.2. STOC analysis was performed off-line using IGOR Pro and Excel software. A STOC was identified as a signal with at least three times the BK_{Ca} single channel current amplitude.

3.5 Materials.

Fluo-4-AM was purchased from Molecular Probes (Eugene, OR, USA). All salts and other drugs were obtained from Sigma-Aldrich (Munich, Deisenhofen or Schnelldorf, Germany) or Merck (Darmstadt, Germany). In cases where DMSO was used as a solvent, the maximal DMSO concentration after application did not exceed 0.5%.

3.6 Statistical analysis.

Data are presented as means ± SEM. Statistically significant differences in mean values were determined by Student's unpaired t-test or one-way analysis of variance (ANOVA). *P*-values < 0.05 were considered statistically significant; “n” represents the number of cells.

4. Results

4.1 CaveolarT-type $Ca_V3.2$ channels contributeto Ca^{2+}sparks

We first studied the contribution of $Ca_v3.2$ channels to generation of Ca^{2+} sparksby using 50 µmol/L Ni^{2+}, which selectively blocks $Ca_v3.2$ channels in VSMCs. Ca^{2+} sparks **(Figure1)** were measured in Fluo-4-AM–loaded VSMCs in the absence (control) or presence of Ni^{2+}.Ni^{2+} decreased the Ca^{2+} spark frequency in mesenteric artery VSMCs (0.085±0.01 Hz, control vs. 0.046±0.01 Hz, with Ni^{2+}, P<0.05). In contrast, Ni^{2+} failed to decrease Ca^{2+} spark frequency in tibial or cerebral VSMCs. These data suggest that the contribution of T-type $Ca_V3.2$ channels to Ca^{2+} spark generation varies according to the vascular bed from whichVSMCs originate.

Caveolae are submicroscopic, plasma membrane pits that are rich in cholesterol and sphingolipids. Decreasing the cholesterol level could disrupt the pits structure to destruct the caveolar ion channels [9]. We used methyl-ß-cyclodextrin, a cholesterol-depleting drug, to unfold caveolae to explore the functional relationship between the caveolar domain and $Ca_v3.2$ channels. Isolated mesenteric artery VSMCs were initially exposed to Ni^{2+} (50 µM) or incubated with methyl-ß-cyclodextrin (10 mM). Ni^{2+} and methyl-ß-cyclodextrin decreased the Ca^{2+} spark frequency and the fraction of cells with sparks by ~30%. In addition thatapplication of Ni^{2+} to mesenteric artery VSMCs treated withmethyl-ß-cyclodextrin did not further reduce Ca^{2+} spark events.

We applied the same experimental protocols as for isolated VSMCs to mesenteric artery segments. The basic outcome (reduction of Ca^{2+} sparks frequency and percentage of cells developing sparks with Ni^{2+} or with methyl-ß-cyclodextrin, no additional block with the combined presence) was similar to the results gained with isolated VSMCs. Either drug alone or in combination failed to impact Ca^{2+} spark amplitude. Cumulatively, these results suggest that caveolar $Ca_V3.2$ channels trigger Ca^{2+} sparks in mesenteric artery VSMCs.

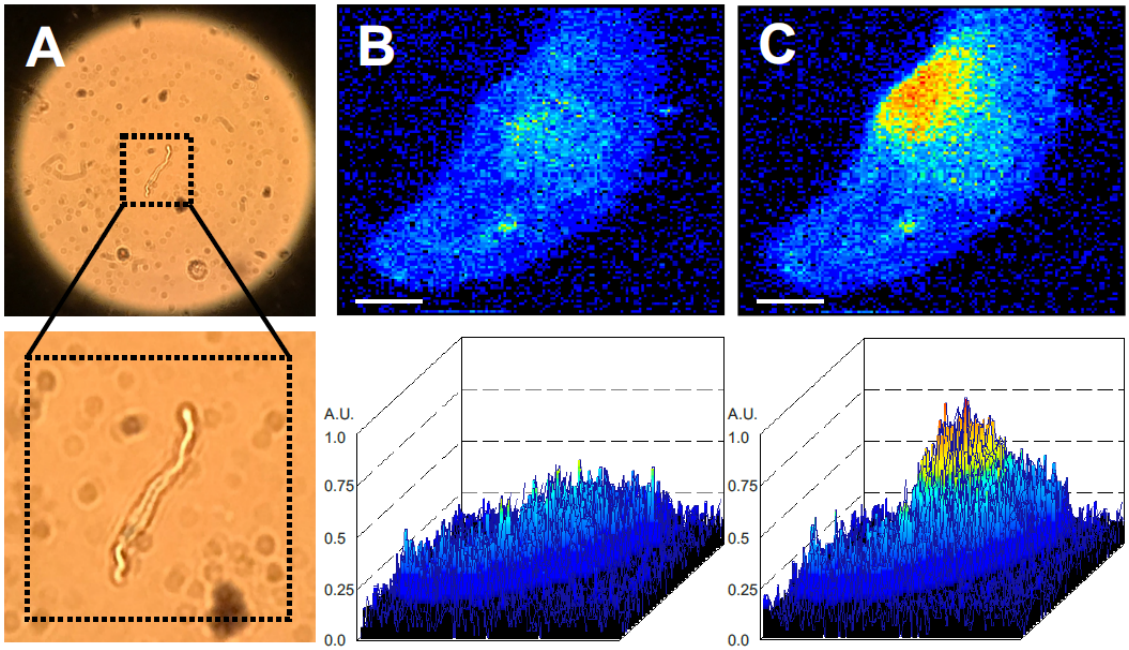

Figure 1: Ca^{2+} spark imaging. (A) Isolated mesenteric artery smooth muscle cell (VSMC). (B) Ca^{2+} fluorescence image of a Fluo-4-AM–loaded mesenteric VSMC. (C) Ca^{2+} fluorescence image of the samecell as in B during the occurrence of an elementary Ca^{2+} release event (Ca^{2+} spark).

4.2 Individual contributions of $Ca_V3.2$ and $Ca_V1.2$ channels to Ca^{2+}sparks

Individual contributions of $Ca_v1.2$ channels and $Ca_v3.2$ channels on Ca^{2+} spark generation in mesenteric arterieswere studied by using wild-type mice and the Cav1.2 blocker Cd^{2+} (200µM) [6]. As previously observed [6], block of Cav1.2 reduced Ca^{2+} sparks frequency and the percentage of cells firing by ~70% in mesenteric artery VSMCs; Cd^{2+} did not affect Ca^{2+} spark amplitude [6]. We next studied the role of $Ca_V3.2$ channels in triggering those remaining sparks and found that both Ni^{2+} and methyl-ß-cyclodextrin completely abolished Ca^{2+} spark events in VSMCs treated with Cd^{2+}.

Subsequent experiments revealed that additional application of Ni^{2+} or methyl-ß-cyclodextrin completely abolished the Cd^{2+}-insensitive spark events. To confirm the results on the individual role of T-type$Ca_v3.2$ channels with a genetic knock-out mouse model we used VSMC-specific $Ca_v1.2$ channel gene inactivation (SMAKO) mice[6]. Ca^{2+} spark frequency and the percentage of cells firing Ca^{2+} sparks was diminished in mesenteric VSMCs of SMAKO mice. Ni^{2+} completely abolished Ca^{2+} spark events in SMAKO mice.The unfolding of caveolae by methyl-ß-cyclodextrin in SMAKO mice also completely inhibited Ca^{2+} sparks. $Ca_v3.2$ housed in the pits structure of caveolae is a regulator of Ca^{2+} spark generation in mesenteric arteries.These data mimic the results with T-type channel block by Ni^{2+} described above in wild-

type mice in presence of Cd^{2+}.Furthermore, Cd^{2+} had no effect on the frequency and percentage of cells firing Ca^{2+} sparks in SMAKO.

4.3 RyR2s in local calcium release in VSMCs

We confirmed our results using transgenic mouse line in whichCre-recombinase expression is driven by the SM myosin heavy chain promoter.Reverse transcription–polymerase chain reaction showed that RyR2 mRNA was almost undetectable in mesenteric and tibial arteries, of SM myosin heavy chain–$Ryr2^{-/-}$ mice compared with control mice. Mesenteric artery SMCs from these mice lacked Ca^{2+} sparks. By contrast,Ca^{2+} sparks were present in 28% of VSMCs fromcontrol mice (n=127).Additionalexperiments performed in SMCs from tibial arteriesshowed that 55.3% of wild-type (control) cells exhibited Ca^{2+}sparks, albeit at a reduced frequency(0.06±0.01 Hz) compared with VSMCs from wild-type littermates of SM-$Ryr2^{-/-}$ mice.

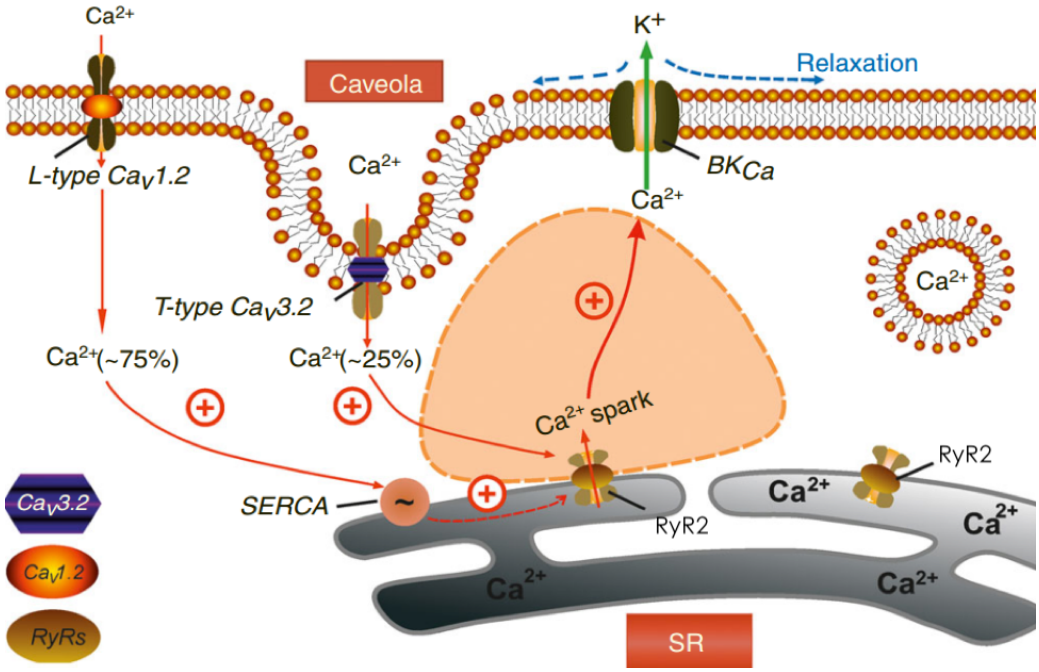

Figure 2. Proposed model of the role of $Ca_V1.2$ and $Ca_V3.2$ channels in Ca^{2+} sparks generation. Ca^{2+} sparks are produced by opening of clustered ryanodine receptor-2 (RyR2) in the SR, which produce a negative-feedback effect on vasoconstriction. This vasodilatory effect is mediated by activation of large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, which results in hyperpolarization of VSMCs and reduced global cytosolic [Ca^{2+}]. The majority (~70-80%) of Ca^{2+} sparks is triggered by $Ca_V1.2$ channels contributing to global cytosolic [Ca^{2+}], which in turn

influences luminal SR calcium *via* SERCA. A minority (~20-30%) of Ca^{2+} sparks is generated by local and tight coupling between $Ca_V3.2$ channels in caveolae to initiate Ca^{2+} sparks. $Ca_V3.2$ channels are located in pit structures of caveolae. In line with this model, depletion of caveolae or $Ca_V3.2$ channel blockade similarly decreased Ca^{2+} sparks by ~20%. SR, sarcoplasmic reticulum. VSMC, mesenteric artery vascular smooth muscle cell. SERCA, calcium pump.

5. Discussion

The results of this study support that T-type $Ca_V3.2$ channel,whichlocated in caveolae on cell membrane, contribute to Ca^{2+} sparks generation in mesenteric VSMCs**(Figure 2)**. L-type $Ca_V1.2$ channels are play predominate role in mediating Ca^{2+} influx in VSMCs, SR refilling and consequently Ca^{2+} spark generation [6]. T-type $Ca_V3.2$ channelsrepresent an additional source for generation of mesenteric VSMC Ca^{2+} sparks.Caveolae are important in local Ca^{2+} spark generation and $Ca_V3.2$ channels, contrary to $Ca_V1.2$ channels, are located in caveolae in close apposition to RyRs.Importantly, RyR2 play a critical role in elementary Ca^{2+} signaling.

5.1 VDCC-RyR Signaling and Ca^{2+} spark

5.1.1 $Ca_V1.2$ channels play a predominant role in Ca^{2+} spark generation

In VSMCs, the global cytosolic Ca^{2+} concentration isregulated by membrane potential-dependent Ca^{2+} influx pathways, with a major role of L-type Ca^{2+} channels [6,].Ca^{2+} sparks are elementary Ca^{2+}-release events generated by a single Ca^{2+}-release unit (CRU) composed of a cluster of ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR).Ca^{2+} sparks can be visualized by use of fluorescent,calcium-sensitive dyes (e.g. Fluo-3 /-4/AM) in all typesof muscle, including striated and SMCs.

Ca^{2+} sparks are involved in both positive- and negative-feedback regulation of global cytosolic [Ca^{2+}] to regulate the contraction of smooth muscle cells. Unlike Ca^{2+} influx *via* VDCCs, Ca^{2+} release from the SR in the form of Ca^{2+} sparks paradoxically causes vasodilation in peripheral resistance arteries. There are two reasons for this counterintuitive effect of Ca^{2+} sparks in arterial VSMCs. First, a single spark produces a remarkably high (10–100 μmol/L) local (~1% of the cell volume) increase in $[Ca^{2+}]_i$, but increases global $[Ca^{2+}]_i$ by less than 2 nmol/L. For triggering Ca^{2+} sparks in VSMCs RyRs need to form clusters. At least 10 RyRs open for one Ca^{2+} spark. Second, Ca^{2+} sparks occur in close proximity to the cell membrane, where every Ca^{2+} spark activates numerous, approximately 15, large-conductance Ca^{2+}-sensitive K^+ (BK_{Ca}) channels, causing K^+ efflux. The resultant spontaneous transient outward currents hyperpolarize VSMCs, thereby decreasing Ca^{2+} entry through voltage-dependent Ca^{2+} channels via a deactivation process. The net result of Ca^{2+} spark–BK_{Ca} channel coupling is decreased global $[Ca^{2+}]_i$ in VSMCs resulting in vasodilation. In some smooth muscle, Ca^{2+} sparks may exert a depolarizing effect through opening Cl^- channels [1]. In this regard, TMEM16A Cl^- channels have been proposed to comprise an important pathologic mechanism underlying the vasoconstriction and remodeling of pulmonary arteries. TMEM16A is required for peripheral blood vessel contractility and plays a general role in arteriolar and capillary blood flow [10].TMEM16A is also a key regulator of coronary blood flow and is implicated in the altered contractility of coronary arteries in hypertension [11].

This study provided two further lines of evidence linking L-type $Ca_V1.2$ channels to the generation of Ca^{2+} sparks. First, deletion of the smooth muscle-specific $Ca_V1.2$ channel gene in (SMAKO) mice decreased Ca^{2+} spark frequency by 50%, 80% and 75% in tibial, cerebral and mesenteric arteries, respectively [6]. Second, Cd^{2+}, a selective inhibitor for high voltage-activated L-type $Ca_V1.2$ channels but not low voltage-activated T-type Ca^{2+} channels diminished the frequency of Ca^{2+}sparks by 75%. These data align with previous results and indicate that $Ca_V1.2$ channels act as the primary Ca^{2+} influx pathway responsible for ~70-80% of Ca^{2+} spark events in VSMCs [6].

5.1.2 Role of VDCC-RyR Signaling on Ca^{2+} spark generation in VSMCs

Similarity to the heart, L-type $Ca_V1.2$ Ca^{2+} channels are presumably the predominant pathway by which extracellular Ca^{2+} triggers Ca^{2+} sparks in arterial smooth muscle. In cardiac muscle cells, tight coupling between $Ca_V1.2$/RyR2 seems to underlie CICR to generate Ca^{2+} sparks. Wang et al.[12] determined the coupling fidelity of L-type Ca^{2+} channels and RyR in rat ventricular myocytes with 0.71, i.e. 71% opening events of L-type channels trigger Ca^{2+} sparks. In contrast, RyRs activation is not tightly linked to the opening of L-type Ca^{2+} channels to trigger Ca^{2+} sparks in smooth muscle [6, 13]. The analysis of latencies of calcium sparks appearing after L-type $Ca_V1.2$ channel openings at different membrane potentials [6, 13] revealed a rather loose coupling. Nevertheless, $Ca_V1.2$ channels could be shown to contribute to global cytosolic [Ca^{2+}], which in turn influences luminal SR calcium and thus Ca^{2+} sparks in arterial smooth muscle [6,]. The loose coupling between L-type $Ca_V1.2$ channel and RyRs is presumably due to an increase in the effective distance between both channels, resulting in an uncoupling of the obligate relationship that exists in striated muscle between the action potential and calcium release [13].

The close proximity between Ca_V channels and RyRs for E-C coupling in striated muscle is ensured by placing the L-type Ca_V1 channels inside t-tubules precisely aligned to parts of the SR in skeletal muscle and cardiomyocytes [14]. Also in VSMCs a precise delivery of the Ca^{2+} ions supplied by VDCCs with short diffusion ways to the SR is essential to trigger Ca^{2+} sparks for the indirect regulation of the VSMC contraction state. However, smooth muscle cells do not have t-tubuli. Instead, they possess abundant cell membrane caveolae [8]. Löhn et al were the first to propose that caveolae could represent additional structural elements necessary for the generation of Ca^{2+} sparks in cardiomyocytes and arterial smooth muscle [15]. This concept is supported by findings of Boittin et al.[16], who found RyRs in vicinity of caveolae.Impairments of the caveolae structure, e.g. via cholesterol depletion with methyl-beta-cyclodextrin or by genetically knocking out essential proteins like EHD2 or caveolin 1, reduce the frequency of Ca^{2+} sparks [17, 18] (Fan et al. 2019 (submitted)). We recently also found that BK_{Ca} channels are not the downstream mediators of perivascular adipose tissue-dependentarterial vasorelaxation.

5.2 Caveolar $Ca_V3.2$ channelsandCa^{2+} spark

5.2.1 $Ca_V3.2$ channels have contribution on calcium spark generation

L-type $Ca_V1.2$ is typically thought to play a predominate role in the signaling pathway of VDCCs-RyRs-BK_{Ca} in vascular smooth muscle[6.] Nevertheless, pharmacological or genetic $Ca_V1.2$ channel ablation does not fully eliminate Ca^{2+} spark events and STOCs, RyRs-mediated Ca^{2+} spark-dependent regulation of arterial myogenic tone [6,]. L-type $Ca_V1.2$ channels are not the only voltage-dependent calcium channels expressed in vascular tissue[18].

$Ca_V3.1$ and $Ca_V3.2$ T-type channels have been found in rat and human renal microvessels (preglomerular vessels,juxtamedullary efferent arterioles, and vasa recta) to contribute to control of blood pressure, glomerular filtration rate, and salt and water homeostasis [19,]. $Ca_V3.1$ and $Ca_V3.2$ channels have been identified by RT-PCR, Western blot, immunolocalization or functional studies in cerebral arteries [20], aorta and mesenteric arteries of rats[,21], in human subcutaneous vessels [21] and in mouse cremaster arterioles [22]. T-type channels could contribute to alterations of rat cerebral network perfusion (20-50% of the total response) as biophysical modeling revealed based on data obtained in arterial diameter measurements [20].

T-type calcium channels were originally described as low-voltage-activated (LVA) channels because they can be activated at more negative membrane potentials compared to high-voltage-activated channels, such as $Ca_V1.2$. They are also characterized by their tiny and transient inward Ca^{2+} currents (called T-type currents) resulting from small depolarization. No specific antagonists are available to separate each subtype of T-type channel. Mibefradil, the most commonly used T-type channel blocker, has not only equally function on each of the three T-type channel subtypes [23], but also has been shown to block other ion channels. Low concentrations of nickel (with an IC_{50} value of about 13 μM) have been demonstrated successfully in $Ca_V3.2$ channel blocking, whereas ~20-fold of this IC_{50} concentration was necessary toinhibit $Ca_V3.1$ channels or L-type channels, with IC_{50} values of 250 μM and 324 μM, respectively [24, 25]. Experiments using $Ca_V3.1$ knockout mice indicate that $Ca_V3.1$ channels contribute to the sinoatrial node pacemaker activity and atrioventricular conduction but do not control systemic blood pressure [26]. Also, $Ca_V3.1$ channels appear to be necessary for proper progression of the cell cycle in human pulmonary artery smooth muscle cells [27].However, there are no reports on vascular abnormalities in $Ca_V3.1$ knock-out animals.

Research based on the pharmacological (Ni^{2+}) or/and genetic tools clearly ascertained the contribution of $Ca_V3.2$ in VSMCs.Thosestudies indicate that T-type $Ca_V3.2$ channels could mediate Ca^{2+} influx to trigger Ca^{2+} sparks to cause STOCs affecting the arterial myogenic response [7, 8]. Ca^{2+} sparks and STOCs have been suppressed by ~25% by using $Ca_V3.2$deficient rats. Those results indicate that T-type Ca^{2+} channels could contribute to Ca^{2+} spark generation and consequently BKCa channel activation to elicit negative feedback control of arterial tone [7]. In order to rule out non-specific effects of Ni^{2+} on $Ca_V1.2$ channelmediated

Ca^{2+}sparks generation in VSMCs, we used $Ca_V1.2$ channels blocker, Cd^{2+}, or the genetic ablation of VSMC $Ca_V1.2$ channels (SMAKO mice) before probing Ni^{2+} effects on Ca^{2+} sparks. Our results indicated that Ni^{2+} completely inhibited Ca^{2+} sparks after silencing $Ca_V1.2$ channels. Our data provide firm evidence that T-type channels provide sufficient Ca^{2+} influx on Ca^{2+} sparks generation in the absence of $Ca_V1.2$ channels in mouse mesenteric arteries.But we did not find the same results in tibial and cerebral arteries. Thus, we conclude that $Ca_V3.2$ channels seem to be responsible for 20-30% of the Ca^{2+} spark generationin mesenteric arteries. The relative contribution of L-type vs. T-type channels in triggering Ca^{2+} sparks inVSMCs depends on the membrane voltage of the cells [28]. Since L-type channels are high-voltage (-40~-20mV) activated Ca^{2+} channels, they trigger Ca^{2+} sparks at relatively high membrane potentials. In contrast, T-type channels are low-voltage (-60~-40mV) activated Ca^{2+} channels, which can activate Ca^{2+} sparks only at relative low membrane potentials [28].

5.2.2 Caveolae microdomains and Ca^{2+} sparks

Caveolae are flask-shaped plasma membrane structures found in several cell types, especially in the cardiac, skeletal, and vascularsmooth muscle cells [7, 8]. A number of transport proteins have been identified to reside in caveolae, including Ca^{2+} channels, Na^+ channels, Na^+/Ca^{2+} exchangers. Abundant evidence indicates that caveolae may play a key function in formation of localCa^{2+} sparks and finally to excitation-contraction coupling in smooth muscle cells and cardiac muscle cells.Caveolae are cholesterol and sphingolipid enriched membrane microdomains that form flask-shaped plasma membrane structures in contractile cells including vascular smooth muscle. Cholesterol depletion by methyl-ß-cyclodextrin disrupts these lipid rafts and thus provides a means to assess their functional importance [9, 15, 29].

Löhn et al were the first to propose that caveolae could representadditional structural elements necessary for the generation of Ca^{2+} sparks in cardiomyocytes and arterial smooth muscle[4]. This concept is supported by findings of RyRs in vicinity of caveolae. Impairments of the caveolae structure, e.g. via cholesterol depletion with methyl-beta-cyclodextrin or by genetically knocking out essential proteins like EHD2 or caveolin-1, reduce the Ca^{2+} spark events.T-type $Ca_V3.2$ channels reside inside caveolae [7, 8]. In this study, we applied methyl-ß-cyclodextrin to VSMCs [15], that decreased frequency of sparks and fraction of cells developing sparks. The sparks amplitudes were not affected. These results match the data from VSMCs with $Ca_v3.2$ channels blocked by Ni^{2+}.Subsequent experiments revealed that Ni^{2+} failed to modulate spark events in the VSMCs or intact arteries after methyl-ß-cyclodextrin treatment. A similar reduction has been observed in mesenteric VSMCs lacking caveolin-1 [30].A simple explanation might be that the function of T-type $Ca_v3.2$ channels possibly depends essentially on their localization in intact caveolae.This interpretation aligns with past ultrastructural findings

which used immunogold labeling to highlight that $Ca_V3.2$ channels are associated with caveolae structures [8].

To confirm the importance of caveolae-standing $Ca_v3.2$ channels we further analyzed the impact of methyl-ß-cyclodextrin treatment or $Ca_v3.2$ channel block by Ni^{2+} on VSMCs without functional L-type $Ca_V1.2$ channels (blocked by Cd^{2+} or genetically ablated using SMAKO mice).In all combinations of blocked or absent L-type channels, we failed to observe Ca^{2+} sparks, consistent with T-type $Ca_V3.2$ channels localizing to caveolae and contributing to the generation of Ca^{2+} sparks. Unfolding caveolae by using methyl-ß-cyclodextrin to enlarge the diffusional space between the caveolar Ca^{2+} influx channels and RyRs in CRUs [15], we found that Ca^{2+} spark events triggered by $Ca_V3.2$ channels were completely inhibited.We conclude that a close apposition of $Ca_V3.2$ channels in caveolae to RyRs is crucial for T-type $Ca_V3.2$ to generate Ca^{2+} sparks in mesenteric artery VSMCs. These results suggest that T-type $Ca_V3.2$ channels in caveolar microdomains exhibit tight and locally restricted control of RyRs in CRU via local Ca^{2+} elevations **(Figure 2)**. However, the relevance of these findings is still unknown as the signaling pathway has only been described in cultured VSMCs [30] or in VSMCs of non-mesenteric origin [31]. Noteworthy, Ni^{2+} increased STOC frequency in superior epigastric arteries (SEA) at -30 mV [32], which might contribute to vasoconstriction of this type of blood vessel.

5.3 RyR2 mediate elementary SR calcium events

RyRs are major Ca^{2+}-release channels in the SR membrane of myocytes that contribute to the regulation of contractility. They are large proteins (about 565 kDa), regulated by a multitude of factors and conditions like pH, phosphorylation, oxidation, inorganic ions like Ca^{2+} and Mg^{2+}, the name giving ryanodine, caffeine, nucleotides, neomycin, tetracaine, halothane, isoflurane, dantrolene, imperatoxin, arachidonic acid and many others (for review see e.g. [33]. Boittin et al.[16] found RyRs in SR sections close to the cell membrane. Fritz et al.[34] confirmed that finding for RyR1 in urinary bladder SMCs. However, they detected RyR2 and RyR3 mainly in the deep SR. Although already expressed at birth, RyRs form clusters during ontogeny of arterial smooth muscle cells. These CRUs are a prerequisite to produce Ca^{2+} sparks as elementary Ca^{2+} release events in VSMCs to produce vasodilation in resistance arteries [35].

The ability of drugs to regulate RyRs to generate Ca^{2+}-sparks in VSMCs (portal vein) was demonstrated for caffeine (1 mmol/L) or low doses of ryanodine (1 µmol/L) VSMCs exhibit all three known RyR isoforms (RyR1, -2, -3).Here, we identify RyR2 as the key SR Ca^{2+}-release channelinvolved in generating Ca^{2+} sparks in VSMCs. RyR2 plays a major role in $Ca_V1.x$/RyR coupling incardiac muscle, RyR1 is crucial in skeletal muscle.Although the importance of the $Ca_V1.2$ Ca^{2+} channel forproper function of arterial VSMCs is well established, wepreviously demonstrated that Ca^{2+} spark generation in thesecells does not rely on the intimate association between$Ca_V1.2$ L-type channels and RyRs. Instead, $Ca_V1.2$ channelscontribute to global

cytosolic [Ca^{2+}], which, in turn, influences luminal SR Ca^{2+} and thus Ca^{2+} sparks.In thepresent study, we investigated the contribution of RyR2s toCa^{2+} sparks in arterial VSMCs. We found that RyR2s areessential for the development of Ca^{2+} sparks inVSMCs.

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7. Statutory Declaration

"I, Gang Fan, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic voltage-gated $Ca_V3.2$ channels and Ca^{2+} sparks in mesenteric artery vascular smooth muscle (Spannungsabhängige $Ca_V3.2$-Kanäle und Ca^{2+}-Sparks in der glatten Gefäßmuskulatur der Mesenterialarterie), independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (isolation of arterial vascular smooth muscle cells, calcium imaging measurements, electrophysiology, statistical analysis) and results (Figure 1, 3, 4, 5, 6, 8 in the Journal of physiology. Figure 5 in the Journal of the American Heart Association) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

Declaration of your own contribution to the publications

Gang Fan contributed the following to the below listed publications:

Publication 1: Fan, G., Kaßmann, M., HashadA. M., Welsh D. G. and Gollasch M.(2018). Differential Targeting and Signalling of Voltage-gated T-type $Ca_V3.2$ and L-type $Ca_V1.2$ Channels to Ryanodine Receptors in Mesenteric Arteries.*Journal of Physiology-London*. 596(20): 4863-4877.**Impact Factor (2018): 4.95**

Contribution (please set out in detail): Design of experimental protocols, preparation of isolated smoothmuscle cell, calcium imaging measurements in smooth muscle cell, whole cell patch-clamp measurements in mesenteric smooth muscle cell, analysis and interpretation of data, writing the first draft of the manuscript, interpretation of results, and literature searches. Figure 1, 3, 4, 5, 6 and 8 were created on the basis of my statistical evaluation.

Publication 2: Kaßmann, M.,Szijártó, I.A., García-Prieto, C.F., **Fan, G.,** Schleifenbaum, J., Anistan, Y.M., Tabeling, C., Shi, Y., Noble, F. le, Witzenrath, M., Huang, Y., Markó, L., Nelson, M.T., Gollasch, M. (2019). Role of Ryanodine Type 2 Receptors in Elementary Ca^{2+} Signaling in Arteries and Vascular Adaptive Responses. *Journal of the American Heart Association,* 8(9), e010090. **Impact Factor (2018): 4.66**

Contribution (please set out in detail): Preparation of isolated smoothmuscle cell, calcium imaging measurements in smooth muscle cell, collect data, analysis and interpretation of data. 2019. Figure 5 were created on the basis of my statistical evaluation.

_____

Signature, date and stamp of first supervising university professor / lecturer

_____

Signature of doctoral candidate

8. Selected Publications

8.1 Publication #1

Differential targeting and signaling of voltage-gated T-type $Ca_V3.2$ and L-type $Ca_V1.2$ channels to ryanodine receptors in mesenteric arteries.

Fan, G., Kaßmann, M., HashadA. M., Welsh D. G. and Gollasch M.(2018). Differential Targeting and Signalling of Voltage-gated T-type $Ca_V3.2$ and L-type $Ca_V1.2$ Channels to Ryanodine Receptors in Mesenteric Arteries.*Journal of Physiology-London*. 596(20): 4863-4877.

https://doi.org/10.1113/JP276923

Publication I

Journal Data Filtered By: **Selected JCR Year: 2016** Selected Editions: SCIE,SSCI
Selected Categories: **"PHYSIOLOGY"** Selected Category Scheme: WoS
Gesamtanzahl: 84 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	PHYSIOLOGICAL REVIEWS	25,952	27.312	0.033220
2	Annual Review of Physiology	8,818	11.115	0.012360
3	JOURNAL OF PINEAL RESEARCH	7,278	10.391	0.008040
4	Comprehensive Physiology	2,641	6.949	0.012280
5	PHYSIOLOGY	2,946	6.076	0.006070
6	CELLULAR PHYSIOLOGY AND BIOCHEMISTRY	8,744	5.104	0.014190
7	Acta Physiologica	3,911	4.867	0.009770
8	Reviews of Physiology Biochemistry and Pharmacology	713	4.769	0.000630
9	JOURNAL OF PHYSIOLOGY-LONDON	48,567	4.739	0.047830
10	EXERCISE AND SPORT SCIENCES REVIEWS	2,674	4.431	0.003780
11	International Journal of Behavioral Nutrition and Physical Activity	7,028	4.396	0.020720
12	AMERICAN JOURNAL OF PHYSIOLOGY-LUNG CELLULAR AND MOLECULAR PHYSIOLOGY	13,082	4.281	0.018040
13	JOURNAL OF GENERAL PHYSIOLOGY	7,714	4.200	0.009920
14	AMERICAN JOURNAL OF PHYSIOLOGY-ENDOCRINOLOGY AND METABOLISM	20,249	4.142	0.025980
15	Frontiers in Physiology	7,664	4.134	0.031330
16	JOURNAL OF CELLULAR PHYSIOLOGY	17,632	4.080	0.023490
17	AMERICAN JOURNAL OF PHYSIOLOGY-RENAL PHYSIOLOGY	16,787	3.611	0.022770
18	AMERICAN JOURNAL OF PHYSIOLOGY-CELL PHYSIOLOGY	16,627	3.602	0.019200
19	JOURNAL OF BIOLOGICAL RHYTHMS	2,617	3.500	0.004220
20	AMERICAN JOURNAL OF PHYSIOLOGY-GASTROINTESTINAL AND LIVER PHYSIOLOGY	14,393	3.468	0.020340
21	JOURNAL OF APPLIED PHYSIOLOGY	42,740	3.351	0.030480

8.2 Publication #2

Role of Ryanodine Type 2 Receptors in Elementary Ca^{2+} Signaling in Arteries and Vascular Adaptive Responses.

Kaßmann, M., Szijártó, I.A., García-Prieto, C.F., **Fan, G.,** Schleifenbaum, J., Anistan, Y.M., Tabeling, C., Shi, Y., Noble, F. le, Witzenrath, M., Huang, Y., Markó, L.,Nelson, M.T., Gollasch,M.(2019). Role of Ryanodine Type 2 Receptors in Elementary Ca^{2+} Signaling in Arteries and Vascular Adaptive Responses. *Journal of the American Heart Association,* 8(9), e010090.

https://doi.org/10.1161/JAHA.118.010090

Publication II

Journal Data Filtered By: **Selected JCR Year: 2018** Selected Editions: SCIE,SSCI Selected Categories: **"CARDIAC and CARDIOVASCULAR SYSTEMS"** Selected Category Scheme: WoS

Gesamtanzahl: 136 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	EUROPEAN HEART JOURNAL	57,358	23.239	0.125920
2	CIRCULATION	166,484	23.054	0.211290
3	JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY	100,986	18.639	0.193290
4	Nature Reviews Cardiology	6,301	17.420	0.018820
5	CIRCULATION RESEARCH	52,988	15.862	0.072290
6	EUROPEAN JOURNAL OF HEART FAILURE	13,107	13.965	0.027620
7	JAMA Cardiology	3,280	11.866	0.019320
8	JACC-Cardiovascular Imaging	8,801	10.975	0.026160
9	JACC-Cardiovascular Interventions	11,555	9.544	0.033640
10	JACC-Heart Failure	3,537	8.910	0.016830
11	JOURNAL OF HEART AND LUNG TRANSPLANTATION	12,436	8.578	0.027310
12	CARDIOVASCULAR RESEARCH	21,828	7.014	0.021500
13	European Heart Journal-Cardiovascular Pharmacotherapy	442	6.723	0.001430
14	Circulation-Heart Failure	6,900	6.526	0.022830
15	BASIC RESEARCH IN CARDIOLOGY	4,137	6.470	0.005590
16	PROGRESS IN CARDIOVASCULAR DISEASES	4,055	6.162	0.008860
17	JOURNAL OF THE AMERICAN SOCIETY OF ECHOCARDIOGRAPHY	10,478	6.111	0.016060
18	EUROPACE	10,908	6.100	0.025320
19	Circulation-Cardiovascular Interventions	5,289	6.060	0.016640

Publication II

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
20	Cardiovascular Diabetology	5,392	5.948	0.011550
21	Circulation-Cardiovascular Imaging	5,456	5.813	0.018480
22	European Journal of Preventive Cardiology	4,782	5.640	0.013370
23	CANADIAN JOURNAL OF CARDIOLOGY	6,710	5.592	0.018500
24	JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY	29,599	5.261	0.036950
25	European Heart Journal-Cardiovascular Imaging	5,498	5.260	0.021650
26	HEART RHYTHM	12,344	5.225	0.029030
27	REVISTA ESPANOLA DE CARDIOLOGIA	3,566	5.126	0.004640
28	HEART	18,063	5.082	0.030620
29	JOURNAL OF CARDIOVASCULAR MAGNETIC RESONANCE	5,113	5.070	0.014020
30	JOURNAL OF MOLECULAR AND CELLULAR CARDIOLOGY	14,143	5.055	0.020450
31	Circulation-Arrhythmia and Electrophysiology	6,432	4.968	0.017840
32	Clinical Research in Cardiology	3,022	4.907	0.006760
33	Circulation-Cardiovascular Genetics	3,441	4.864	0.010500
34	Journal of the American Heart Association	13,230	4.660	0.060340
35	TRENDS IN CARDIOVASCULAR MEDICINE	2,667	4.462	0.003930
36	Circulation-Cardiovascular Quality and Outcomes	4,531	4.378	0.014350
37	ATHEROSCLEROSIS	23,442	4.255	0.033500
38	CARDIOVASCULAR DRUGS AND THERAPY	2,109	4.181	0.003140
39	JOURNAL OF NUCLEAR CARDIOLOGY	3,711	4.112	0.004480

Selected JCR Year: 2018; Selected Categories: "CARDIAC and CARDIOVASCULAR SYSTEMS"

9. Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

10. Complete list of publications

Original Publishing

Fan, G., Kaßmann, M., HashadA. M., Welsh D. G. and Gollasch M. (2018). Differential Targeting and Signalling of Voltage-gated T-type $Ca_V3.2$ and L-type $Ca_V1.2$ Channels to Ryanodine Receptors in Mesenteric Arteries.*Journal of Physiology-London*. 596(20): 4863-4877. **Impact Factor (2018): 4.95**

Kaßmann, M., Szijártó, I.A., García-Prieto, C.F., **Fan, G.,** Schleifenbaum, J., Anistan, Y.M., Tabeling, C., Shi, Y., Noble, F. le, Witzenrath, M., Huang, Y., Markó, L., Nelson, M.T., Gollasch, M. (2019). Role of Ryanodine Type 2 Receptors in Elementary Ca^{2+} Signaling in Arteries and Vascular Adaptive Responses. *Journal of the American Heart Association,* 8(9), e010090. **Impact Factor (2018): 4.66**

Fan, G.,Kaßmann, M., Cui, Y.Q., Matthaeus, C., Kunz, S., Zhong, C., Zhu S., Xie, Y., Daumke, O., Huang, Y., Gollasch, M. (2019). Age Attenuates the T-type $Ca_V3.2$-RyR Axis in Vascular Smooth Muscle. *Aging Cell*.In Revision.**Impact Factor (2018): 7.346**

Review

Fan, G., Cui, Y.Q., Gollasch, M., Kaßmann, M. (2019). Elementary Calcium Signaling in Arterial Smooth Muscle. *Channels*. In Press.**Impact Factor (2018): 2.289**

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