GSK-7975A, an inhibitor of Ca^{2+} release-activated calcium channels, depresses isometric contraction of mouse aorta

GSK-7975A, an inhibitor of Ca^{2+} release-activated calcium channels, depresses isometric contraction of mouse aorta.

Sofie De Moudt, Dorien De Munck, Isabelle Coornaert, Paul Fransen

Laboratory of Physiopharmacology, Department of Pharmaceutical Sciences, University of Antwerp, Belgium

Corresponding author: Paul Fransen

Laboratory of Physiopharmacology
Campus Drie Eiken, T2
Universiteitsplein 1
B-2610 Antwerp
Belgium
paul.fransen@uantwerpen.be

Email addresses:

sofie.demoudt@uantwerpen.be

dorien_de_munck@hotmail.com

isabelle.coornaert@uantwerpen.be

Declarations of interest: none

ABSTRACT

GSK-7975A is described to inhibit stromal interaction molecule 1(STIM1)-mediated Ca^{2+} release-activated Ca^{2+} channels ORAI 1, ORAI 2 and ORAI 3 in different cell types. The present study investigated whether isometric contractions of mouse aortic segments were affected by this selective store-operated calcium channel inhibitor. Depending on the way by which Ca^{2+} influx pathways were activated during contraction, GSK-7975A inhibited contractility of mouse aortic segments with different affinity. When contractile effects were induced by depolarization as with elevated extracellular K^+ and opening of voltage-gated calcium channels, the affinity was approximately 10 times lower than when contraction was elicited with Ca^{2+} influx via non-selective cation channels. GSK-7975A may repolarize the aortic smooth muscle cells by inhibiting non-selective cation channels, has no effect on IP_3-mediated phenylephrine-induced phasic contractions or on refilling of the contractile sarcoplasmic reticulum Ca^{2+} store, but has significant effects on non-contractile store-operated Ca^{2+} influx.

Keywords: mouse aorta, isometric contraction, GSK-9795A, sarcoplasmic reticulum, CRAC channel

1. INTRODUCTION

Different Ca^{2+} entry mechanisms are involved in regulating intracellular Ca^{2+} as a crucial mediator of vascular smooth muscle cell (VSMC) function and phenotype. Whereas voltage-gated L-type Ca^{2+} channels (VGCC) contribute to their contractile phenotype, Ca^{2+} entry mechanisms via other channels promote a non-contractile phenotype as occurs in various disease states such as restenosis, atherosclerosis and neo-intima formation (Avila-Medina et al., 2018; Berra-Romani et al., 2008; Kudryavtseva et al., 2013; Ruhle and Trebak, 2013). In response to emptying the sarcoplasmic reticulum (SR) Ca^{2+} stores, calcium-release activated Ca^{2+}(CRAC) or store-operated Ca^{2+} entry (SOCE) channels open. It is generally assumed that this Ca^{2+} influx pathway involves two players: the SR Ca^{2+} sensor (stromal interaction molecule, STIM), and the plasmalemmal Orai channel. STIM senses decreased calcium concentration in the SR and in turn activates the plasmalemmal Orai channel allowing the influx of Ca^{2+} ions (Avila-Medina et al., 2018; Cahalan et al., 2007). VSMC in their contractile status display very low STIM/Orai 1 protein expression and CRAC channel activity (Ruhle and Trebak, 2013). A number of CRAC channel inhibitors, such as 2-APB, also have inhibitory effects on transient receptor potential (TRP) channels, IP_3 receptors and VGCC. Therefore, the search for specific and selective blockers of CRAC channels is still ongoing. Synta-66, BTP2, GSK-7975A and GSK-5503 have been proposed as selective CRAC (Orai 1-3) channel blockers (Derler et al., 2013b; Zhang et al., 2020). Of these, GSK-7975A inhibits mediator release from mast cells and pro-inflammatory cytokine release from T cells in a variety of species (Rice et al., 2013), Ca^{2+} waves and contractions in urethral smooth muscle cells (Drumm et al., 2018) and currents through Orai 1, 2 and 3 channels expressed in HEK293 cells (Zhang et al., 2020). The affinity for VGCC (IC_{50} = 8 μM) (or different TRP channels, recombinantly expressed in HEK cells, IC_{50} > 10 μM) was lower compared with ORAI 1 and ORAI 3 currents (IC_{50} = 4 μM) (Derler et al., 2013b).

The present study investigated whether isometric contractions of mouse aortic segments are inhibited by GSK-7975A; and whether this inhibition was due to blocking Ca^{2+} influx induced by the SR store emptying. Isometric contractions induced by α_1 adrenergic stimulation of mouse aortic segments as with phenylephrine involve the release of Ca^{2+} from IP_3-sensitive SR Ca^{2+} stores and Ca^{2+} influx via VGCC and non-selective cation channels (NSCC) (Fransen et al., 2015; Leloup et al., 2015a). Although VGCC are the main determinants of isometric contraction of mouse aortic segments, the contribution of NSCC Ca^{2+} influx to the contraction is

not negligible especially after inhibition of basal NO release with L-NAME (Leloup et al., 2017). After emptying the SR Ca^{2+} store with a high concentration of phenylephrine, contractile store refilling in mouse aortic segments is very dependent on Ca^{2+} influx via VGCC (Leloup et al., 2015a), but at present it is unknown whether Ca^{2+} influx via NSCC also contributes to store refilling. In the present study, it is hypothesized that GSK-7975A inhibits channels contributing to SR store refilling in VSMC and, thereby, affects contractile properties of mouse aorta segments.

2. MATERIALS AND METHODS

2.1. Animals

The studies were approved by the Ethical Committee of the University of Antwerp (2015-52), and conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). C57Bl/6 mice (n = 43, food and water ad libitum, 12/12 light-dark cycle) were used at the age of 4 to 7 months. After anaesthesia (sodium pentobarbital, 250 mg/kg, i.p.) and killing the animals by perforating the diaphragm, the thoracic aorta was carefully removed, stripped of adherent tissue and dissected systematically in segments of 2 mm width (6 segments). Vessels were immersed in Krebs Ringer solution (KR solution, 37°C, 95% O_2/5% CO_2, pH 7.4) with (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, CaEDTA 0.025, and glucose 11.1.

2.2. Isometric contractions

Aortic segments were mounted at 16 mN preload in 10 ml organ chambers as previously described (De Moudt et al., 2017). They were rotated among the different (total number, 6) organ chambers to avoid intersegmental differences. Isometric force (EMKA Technologies, France) was acquired with a Powerlab data acquisition system and Labchart software and was reported in mN. In all experiments, endothelial cells were present, but in some experimental conditions, basal NO formation was inhibited with 300 µM N^W-nitro-L-arginine methyl ester (L-NAME) (Leloup et al., 2015b). To avoid any vasomotor interference due to prostanoids, indomethacin (10 mM) was always present. High K^+-solution was prepared as KR solution, in which NaCl was replaced by KCl in equimolar concentrations. Ca^{2+}-free solution (0Ca) was prepared by omitting Ca^{2+} from the KR solution and adding 1 mM EGTA as chelator. To restore 2.5 mM Ca^{2+} KR solution again, 3.5 mM Ca^{2+} was added to 0Ca (0Ca/+Ca condition).

2.3. Concentration-effect curves

Concentration-effect curves for GSK-7975A were constructed in organ chambers (number of organ chamber between brackets): 300 µM L-NAME to inhibit eNOS (2, 4 and 6), 10 µM cyclopiazonic acid to stimulate CRAC channel-mediated Ca^{2+} influx (1 and 2), 1 µM levcromakalim to repolarize the smooth muscle cells to the equilibrium potential for K^+ ions and

prevent Ca^{2+} influx via VGCC (5 and 6). After 20 min equilibration, segments were pre-contracted with 2 µM phenylephrine (1, 2, 5 and 6) or 50 mM K^+ (3 and 4). After 10 to 15 min GSK-7975A was added in cumulative concentrations from 0.03 µM to 100 µM to cause relaxation of the pre-contracted segments. Concentration-response curves were fitted with sigmoidal concentration-response equations with variable slope, which revealed maximal contraction or relaxation responses (E_{max}) and the concentration (K^+) or negative logarithm of the concentration resulting in 50% of the maximal contraction or relaxation (EC_{50} for K^+ and pEC_{50}).

2.4. SR store refilling

Contractile SR store refilling was investigated in the presence of L-NAME (see Fig. 1). After challenging the aortic segments for 3 min with 0Ca/1 mM EGTA solution, 2 µM phenylephrine was added to elicit the first phasic contraction (PE1). After PE1, vehicle (1), 10 µM GSK-7975A (2) or 3.5 µM diltiazem (3) was added. Subsequent re-addition of 3.5 mM Ca^{2+} in the presence of phenylephrine, caused a tonic contraction during which re-filling of the phenylephrine-sensitive contractile Ca^{2+} store occurred. After 5 min, the segment was again challenged with 0Ca/1 mM EGTA for 3 min and a second phasic contraction with 1 µM phenylephrine (PE2) was elicited. The relative amount of the PE2 with respect to the PE1 contraction determines the contractile store re-filling during the 0Ca/+Ca period between PE1 and PE2.

2.5.Basal NO production

We have shown previously that the contraction induced by phenylephrine is time-dependent because of the loss of basal NO production in isometric conditions (van Langen et al., 2012). The effect of GSK-7975A on this time-dependent loss of basal NO efficacy was investigated by studying the contraction by 2 µM phenylephrine at hourly time points from 1 up to 6h with 1 µM levcromakalim to inhibit VGCC Ca^{2+} influx (3 and 4), and 10 µM GSK-7975A (2 and 4).

2.6. Depolarization-induced contractions

The effects of GSK-7975A on depolarisation-induced contraction were investigated by depolarizing the segments consecutively with 10, 15, 20, 25, 30, 35, 40 and 50 mM K^+ in the absence (1 and 2) or presence (3 and 4) of 300 µM L-NAME and in the absence (1 and 3) and presence (2 and 4) of 10 µM GSK-7975A.

2.7.Combined assay of isometric tension and VSMC Ca^{2+}

In myograph experiments, the endothelium was mechanically removed by rubbing the interior of the segment with a braided silk wax to remove interference of endothelial Ca^{2+} signals with the VSMC cytosolic Ca^{2+} assay (Van Hove et al., 2009). The Fura-2 AM (10 µM)-loaded segment was continuously perfused with KR (37 °C) which was aerated with 95 % O_2/5 % CO_2 (pH 7.4). The single emission (510 nm) ratio at dual excitation (340 and 380 nm) was used as a relative measure of free $[Ca^{2+}]_i$ (relative units, RU) and was analysed with Felix software (PTI, USA). Tension was measured simultaneously, acquired at 1 Hz and reported in mN mm^{-1} (Van Hove et al., 2009).

2.8. Statistics

All results are expressed as mean±S.E.M..; n represents the number of mice. Two-way ANOVA analysis with Bonferroni multiple comparison post-hoc test, one-way ANOVA analysis with Dunnett's multiple and paired or unpaired *t*-test (GraphPad Prism, version 9, GraphPad Software, San Diego California USA) were used to compare means of the different experimental groups. A 5% level of significance was selected.

2.9. Reagents

Sodium pentobarbital (Nembutal®) was obtained from Sanofi (Belgium), indomethacin from Federa (Belgium), L-NAME from Sigma (Belgium), diltiazem hydrochloride, levcromalkalim from TOCRIS (United Kingdom), GSK-7975A was a kind gift of Malcolm Begg (Transfer Material Agreement, April 12, 2013, GlaxoSmithKline Research & Development limited).

3. RESULTS

3.1. GSK-7975A inhibits contraction of murine aortic segments by affecting VGCC and non-selective cation channels with different sensitivity.

The concentration-dependent relaxing effects of GSK-7975A were studied in mouse aortic segments in three conditions: (1) depolarized with elevated external K^+ to cause VGCC-mediated contractions (Fransen et al., 2012b); (2) precontracted by 2 µM phenylephrine and eliciting a phasic contraction together with a tonic contraction by Ca^{2+} entry via VGCC and non-selective cation channels (NSCC) (Fransen et al., 2015; Leloup et al., 2015a); and (3) pre-contracted with 2 µM phenylephrine in the absence of extracellular Ca^2 (0Ca) followed external Ca^{2+} replenishment (0Ca/+Ca) to separate phasic and tonic contractions (see protocol **Fig. 1A-C**). Representative examples of concentration-relaxation curves for these contractions in the absence (black) and presence (red) of eNOS inhibition with 300 µM L-NAME are shown in **Fig. 1 (D-F).**

Isometric contractions by depolarization with 50 mM K^+ were inhibited by GSK-7975A with logIC_{50} of -4.98 logM (n=3) (**Fig. 1G**). The IC_{50} was not affected by inhibiting basal NO release from endothelial cells with L-NAME (-4.92 logM, $p>0.05$) or by depolarization of the segments to 0 mV with 124 mM K^+ (the presumable reversal potential for non-selective cation channels) in the absence (-4.64±0.03 logM, n=5, $P>0.05$ versus $50K^+$) or presence of L-NAME (-4.59±0.05, n=5, $P>0.05$ versus 50 K^+, **Fig. 2D).** Addition of 2 µM phenylephrine causes contractions, which were inhibited by GSK-7975A with IC_{50} of -5.55±0.13 logM before eNOS inhibition and with IC_{50} of -4.85±0.14 (n=8, $P<0.05$ versus absence L-NAME) after inhibition of basal NO release with L-NAME (**Fig. 1E, H**). Addition of 2 µM phenylephrine in 0Ca completely emptied the contractile, phenylephrine-sensitive SR Ca^{2+} store (Leloup et al., 2015) (**Fig. 1F**). The tonic contraction by re-addition of extracellular Ca^{2+} was inhibited by GSK-7975A with IC_{50} of -5.33±0.11 logM in the absence and -4.92±0.20 log M ($P<0.05$, n=3) in the presence of L-NAME **(Fig. 1 I).** Hence, when only VGCC Ca^{2+} influx contributed to the contraction (as with 50 or 124 mM K^+, **protocol A, Fig. 1**), the IC_{50} of GSK-7975A was about 12 µM and was NO-independent. GSK-7975A-mediated inhibition of contractions by α_1-

adrenergic stimulation of mouse aortic segments with phenylephrine, which involves as well NSCC as VGCC activation (**protocol B and C, Fig. 1**), were endothelium-dependent and the IC_{50} changed from about 12 µM in the presence of L-NAME, identical to the IC_{50} for inhibition of VGCC channels, to around 5 µM in the presence of basal NO release. To investigate this further, experiments in which the relative contribution of both Ca^{2+} influx pathways to the contraction by phenylephrine was modulated were performed and the relaxation by GSK-7975A was studied.

To promote the contribution of NSCC Ca^{2+} influx to the phenylephrine-induced contraction, two experimental conditions were explored, both in the presence of 300 µM L-NAME (**Fig. 2A**). In one condition the contraction by phenylephrine was elicited in the presence of the SERCA inhibitor cyclopiazonic acid. We have shown before (Fransen et al., 2015) that in the presence of 10 µM cyclopiazonic acid (CPA), the relative contribution of NSCC to the contraction increased from 40 to 60%. In this situation the GSK-7975A inhibition curve significantly shifted to higher sensitivity and the IC_{50} of GSK-7975A changed from -4.85±0.15 (n=8) to -5.35±0.11 (n=5) (**Fig. 2B-D**). In a second condition the activation of VGCC was completely avoided by repolarization of the VSMC of aortic segments with 1 µM levcromakalim (Fransen et al., 2015). Hence, the contraction by phenylephrine was only due to NSCC Ca^{2+} influx. The inhibition curve further shifted to higher sensitivity and IC_{50} was -5.92±0.06 logM (1.2 µM) (**Fig. 2B-D**). In **Fig. 2D,** the log(IC_{50}) is compared between the different conditions. According to these data GSK-7975A has about 10 times higher sensitivity for inhibition of contractions due to NSCC Ca^{2+} influx than to VGCC Ca^{2+} influx.

3.2. Role of GSK-7975A in SR Ca^{2+} store refilling after α_1-adrenergic stimulation with phenylephrine

Contractile IP_3- or phenylephrine-sensitive stores are completely emptied when 2 µM phenylephrine is added 3 min after changing to a calcium-free extracellular solution (Leloup et al., 2015a). Re-filling of this contractile store was studied as shown in the experimental procol of **Fig. 3A.** Data are presented in Fig. 3 C-I. In control conditions, the second phasic phenylephrine contraction (PE2) was similar to the first (PE1) and the +Ca contraction after PE2 was not

different from the tonic contraction measured before the test protocol. As expected, PE1 before addition of GSK-7975A or diltiazem was not different from PE1 in control (**Fig. 3C, F, I).** During the 0Ca/+Ca period between PE1 and PE2, SR stores can be refilled by external Ca^{2+} influx via VGCC and NSCC. When compared with control PE2, PE2 contractions in the presence of GSK-7975A were, however, not signidficantly attenuated, whereas the phasic contractions in the presence of diltiazem were almost completely inhibited (**Fig. 3 D, G, J**). Nevertheless, both GSK-7975A and diltiazem were effective because they significantly reduced the tonic contraction by PE (**Fig. 3 E, H, K**).

In control conditions, the area under the curve of the phasic contractions was significantly depressed for PE2 compared with PE1, indicating that 5 min +Ca is not long enough to cause complete refilling of the contractile stores (**Fig. 3B**). Also in the presence of GSK-7975A, AUC of PE2 was depressed compared with PE1, but was not significantly different from control. Inhibition of VGCC with 3.5 µM diltiazem during the preceding 0Ca/+Ca period caused attenuation of AUC of PE2, suggesting that VGCC majorly contribute to the refilling of phenylephrine-sensitive Ca^{2+} stores.

3.3. GSK-7975A and VGCC-mediated contractions

Because of the high affinity of GSK-7975A for NSCC, it may repolarize the VSMC membrane potential in basal conditions. This repolarizing effect should be reflected in K^+-contraction curves similarly as for levcromakalim. We have previously shown that repolarization of the VSMC membrane potential with the ATP-dependent K channel ($K_{(ATP)}$) opener levcromakalim shifted the K^+-contraction curve to higher K^+ concentrations (less sensitive to depolarization) (Fransen et al., 2012a). Isometric contractions were measured at increasing K^+ concentrations in the absence and presence of L-NAME (inhibition of NO production) and in the absence and presence of 10 µM GSK-7975A (**Fig. 4 A**). This concentration of GSK-7975A inhibits NSCC by more than 50% and VGCC by less than 50% (**see Fig. 1 and 2**). 10 µM GSK-7975A caused significant inhibition of the contraction at 50 mM K^+ by 34±10% in the absence and by 19±12% in the presence of L-NAME, confirming the lower affinity for VGCC (**Fig. 4B**). L-NAME causes large shifts of the window contraction curves (-6.3±0.3 mM K^+, n=4), which is compatible with its depolarizing effect (**Fig. 4C**).

Importantly, and according to its probable NSCC blocking activity, GSK-7975A causes significant shifts of the K^+-contraction curves in the opposite direction of L-NAME (**Fig. 4C, D**). The shifts amounted 3.4±0.9 mM K^+ in the absence of L-NAME (n=4) and 5.1±0.8 mM K^+ in the presence of L-NAME (n=5, p=0.18 vs absence L-NAME). As such, the action of GSK-7975A on voltage-dependent contractions resembles the rightward shifts of the K^+-contraction curves as with exogenous NO or as with levcromakalim (Fransen et al., 2012a).

3.4. Effects of GSK-7975A on NO efficacy following α_1-adrenergic stimulation with phenylephrine.

As suggested by the data in Fig. 4, blocking NSCC-mediated cation influx with GSK-7975A may cause hyperpolarization of the VSMC of aortic rings, thereby mimicking the hyperpolarizing action of basal NO release (Fan et al., 2007; Krippeit-Drews et al., 1992). Because basal NO efficacy is compromised with time after mounting the segments, the contractions by α_1-adrenoceptor stimulation of mouse aortic segments with phenylephrine increase with time (van Langen et al., 2012). This evoked the question whether blocking NSCC with GSK-7975A could prevent the increase of contraction due to the loss of basal NO efficacy with time after mounting (**Fig. 5 A, B**).

Fig. 5 shows the isometric contractions by 2 µM phenylephrine at hourly time intervals after mounting the segments. As expected, in control conditions (KR**, Fig. 5C**), the steady-state contraction elicited by 2 µM phenylephrine increased in an hourly manner between 1 and 6 h. As shown **in Fig. 5G**, the increase of the contraction with time after mounting displayed an exponential time course and contraction increased with a time constant of 1.5h. Incubation of the segments with 10 µM GSK-7975A (**Fig. 5 D**) inhibited and retarded the time-dependent increase of the phenylephrine-induced contraction significantly and the time constant of force increase was approximately 5.5h (**Fig. 5G**). In the presence of 1 µM levcromakalim (**Fig. 5E),** hence, in the absence of VGCC-mediated contractions, phenylephrine-induced contractions were more strongly inhibited and the time constant of force increase with time after mounting further increased to 13.8h (**Fig. 5G**). The tonic contractions remaining in the presence of levcromakalim were all due to Ca^{2+} influx via NSCC, and could be effectively inhibited by 10 µM GSK-7975A (lev + GSK curve, **Fig. 5F**). What remained of the phenylephrine-elicited contractions in the presence of levrcromakalim and GSK-7975A are transient, phasic contractions, which also

increased with time after mounting and which resemble IP_3-mediated contractions induced by phenylephrine in the absence of extracellular Ca^{2+} (Fransen et al., 2015).

The fact that we did observe a trend to slower time-dependent increase of contraction by phenylephrine in the presence of 10 µM GSK-7975A (**Fig. 5G**) is most probable due to the high variability of the steady-state contractions in this condition. In **Fig. 5H**, these effects are shown for the different conditions and it is remarkable that the contraction by phenylephrine is inhibited by pre-incubation with 10 µM GSK-7975A with higher than normal variability. After 6 h, phenylephrine-induced contractions were not inhibited in 2 mice, partly inhibited in 2 mice and completely inhibited in 3 mice. Remarkably, in the presence of GSK-7975A, the highest contractions by phenylephrine at 1h were these contractions that were not inhibited with GSK-7975A at 6h and the lowest contractions at 1h were these that were most inhibited at 6h.

Finally, after 6h the aortic segments in the different conditions were treated with 300 µM L-NAME to inhibit remaining basal NO release, and, subsequently, with 3.5 µM diltiazem to estimate the contribution of VGCC to phenylephrine-induced contraction **(Fig. 6**). Three conditions were studied (**Fig. 6A**): contribution of VGCC and NSCC to the contraction, contribution of VGCC to the contraction (presence of GSK-7975A to block NSCC) and contribution of NSCC to the contraction (presence of levcromaklim to prevent activation of VGCC. In KR, L-NAME caused a further increase of force after 6h, indicating that the phenylephrine-induced contraction was still substantially depressed by basal NO release. Addition of diltiazem inhibited force by approximately 57%. Pre-incubation of the segments with 10 µM GSK-7975A inhibited the phenylephrine-induced contraction in the absence and presence of L-NAME, but L-NAME still increased force after 6h. This force was inhibited by 86±1% with diltiazem, indicating that the contraction by phenylephrine in the presence of L-NAME and GSK-7975A is almost completely due to VGCC Ca^{2+} influx. 1 µM levcromakalim prevented the activation of VGCC during phenylephrine-induced contractions by repolarizing the VSMC, and caused significant depression of the isometric contractions in the absence and presence of L-NAME and, also in this condition, force after 6 h increased with L-NAME. The latter force increase was, as expected, not inhibited with diltiazem and represents force increase by NSCC Ca^{2+} influx.

3.5. GSK-7975A and contractile/non-contractile Ca^{2+} stores.

Previously, we have shown that emptying cytoplasmic SR Ca^{2+} stores by inhibition of the sarco-endoplasmic reticulum Ca^{2+} pump (SERCA) with cyclopiazonic acid caused substantial Ca^{2+} increase without concomitant force increase, which led us to hypothesize that contractile and non-contractile Ca^{2+} stores co-exist in aortic smooth muscle cells (Fransen et al., 2015) (**Fig. 7 C**). To study the effects of GSK-7975A on these non-contractile Ca^{2+} stores, aortic segments were loaded with the Ca^{2+} sensitive fluorochrome Fura-2 to measure intracellular Ca^{2+} simultaneously with contraction of the VSMC, as shown **in Fig. 7 A, B**.

Removal of extracellular Ca^{2+} caused intracellular Ca^{2+} and tension to decrease. Emptying the cyclopiazonic acid-sensitive Ca^{2+} stores with 10 µM cyclopiazonic acid caused transient increase of Ca^{2+} without any tension changes. Subsequent re-addition of extracellular Ca^{2+} caused parallel intracellular Ca^{2+} and tension increase, which for the Ca^{2+} signal were significantly larger than for depolarization with 50 mM K^+ but for the tension significantly smaller. Inhibition of VGCC with 3.5 µM diltiazem caused a slight decrease of intracellular Ca^{2+} without affecting tension, which was transiently increased by re-addition of external Ca^{2+} to the 0Ca/cyclopiazonic acid solution. The Ca^{2+} signal was almost completely blocked by 10 µM GSK-7975A. Results of these experiments indicate that the affinity of GSK-7975A for Ca^{2+} influx channels activated by emptying the non-contractile Ca^{2+} store is larger than for Ca^{2+} channels activated by emptying the contractile Ca^{2+} store.

4. DISCUSSION

Depending on the contractile agent and on the way by which Ca^{2+} influx pathways are activated during contraction, the "selective" Orai channel inhibitor, GSK-7975A, depresses contractility of mouse aortic segments with different affinity. When contractile effects were induced by depolarization as with elevated extracellular K^+ and opening of VGCC, the affinity was approximately 10 times lower than when contraction was elicited via NSCC mediated Ca^{2+} influx. GSK-7975A shifted the K^+ concentration-contraction relationship to higher K^+ concentrations, suggesting that GSK7975A may repolarize the VSMC by inhibiting NSCC. GSK-7975A had no effect on IP_3-mediated phenylephrine-induced phasic contractions or on refilling of the contractile SR Ca^{2+} store, but had significant effects on non-contractile store-operated Ca^{2+} influx.

4.1. GSK-7975A inhibits contraction of murine aortic segments by affecting VGCC and NSCC with different sensitivity

It has already been demonstrated that the specific Orai channel blocker, Synta-66 (5 µM), has no effect on the α_1-adrenoceptor-mediated signalling mechanisms, Ca^{2+} handling in contractile cells or on the contractile machinery of mouse aortic rings (Li et al., 2011). Synta-66 potently inhibited VSMC migration (0.1 µM) and modestly attenuated VSMC proliferation (5 µM), suggesting that its affinity for contractile Ca^{2+}influx pathways was much lower than for non-contractile Ca^{2+} movements. The present study confirmed these findings for GSK-7975A.

In rat coronary VSMC, endothelin-1 mediated constriction and concomitant Ca^{2+} signal were abolished by GSK-7975A and nifedipine, which could be due to co-activation by endothelin-1 of co-localized VGCC, TRPC1 and Orai 1 channels in the plasmalemma of the VSMC (Calderon-Sanchez et al., 2020). Isometric contractions of mouse aortic segments induced by depolarization with elevated K^+ and activation of VGCC Ca^{2+} influx were inhibited with an IC_{50} of 10-30 µM. This value agrees well with the value of 8 µM for inhibition of L-type Ca^{2+} channels, which were recombinantly expressed in HEK cells (Derler et al., 2013a). After α_1-adrenergic stimulation with phenylephrine in the presence of L-NAME, hence after inhibition of basal NO release, the VSMC are depolarized and the IC_{50} was comparable to the IC_{50} for inhibition of VGCC after depolarization with high K^+. By increasing the relative

contribution of NSCC to the contraction from 40 to 60% in the presence of 10 µM cyclopiazonic acid (Fransen et al., 2015), the IC_{50} of GSK-7975A changed from 14 µM to 4.4 µM, suggesting that GSK-7975A has a higher affinity for NSCC than for VGCC mediated Ca^{2+} influx. In the absence of L-NAME, hence the presence of repolarizing basal NO release, the IC_{50} was also decreased from 12 to 2-5 µM. To investigate the affinity for NSCC further, experiments in which the relative contribution of NSCC to the contraction by phenylephrine was maximized, were performed by incubating the aortic segments with levcromakalim. Levcromakalim causes repolarization of the VSMC membrane potential to the K^+ equilibrium potential by activating ATP-dependent K^+ channels and by preventing the activation of VGCC in basal conditions. With electrophysiological techniques we showed previously that aortic segments had a mean resting membrane potential of -60.1±2.6 mV, which was dependent on the extracellular K^+ concentration, and hyperpolarized from -64 to -86 mV following addition of 200 nM levcromakalim, confirming its K^+ channel agonism (Fransen et al., 2012b). GSK-7975A inhibited the NSCC mediated contraction with an IC_{50} of 1.2 µM, which is about ten times lower than the IC_{50} for VGCC inhibition, but which is also lower than the IC_{50} of 4 µM for inhibition of Orai 1 and Orai 3 currents (Derler et al., 2013b).

4.2.SR store refilling

Orai1 channels are typically activated upon depletion of IP_3-sensitive SR Ca^{2+} stores as also occurs with α_1 adrenergic stimulation of vascular smooth muscle cells, but seem to play only a minor role in VSMC contractility (Avila-Medina et al., 2018; Ruhle and Trebak, 2013). Because CRAC channels are believed to be activated by emptying of the SR Ca^{2+} store, it was evident to explore the SR store refilling inhibitory effect of GSK-7975A in the aortic segments. When VSMC IP_3-sensitive contractile SR Ca^{2+} stores were first completely emptied with 1 µM phenylephrine and then refilled by adding extracellular Ca^{2+} in the absence and presence of 10 µM GSK-7975A, a concentration which causes complete inhibition of NSCC (near 100 %) and partial inhibition of VGCC (20-40%), refilling of the contractile SR Ca^{2+} store was minimally affected by GSK-7975A. On the other hand, inhibition of VGCC with 3.5 µM diltiazem caused near-complete inhibition of store refilling. These results suggest that in mouse aortic segments refilling of the contractile SR Ca^{2+} store does not occur via GSK-7975A-sensitive Ca^{2+} permeable channels and confirm that contractile store re-filling mainly occurs via VGCC Ca^{2+}

influx (Leloup et al., 2015a). Eliciting phenylephrine-induced contraction after inhibition of VGCC and NSCC mediated Ca^{2+} influx with respectively levcromakalim and GSK-7975A caused only phasic contractions (Fig. 8), resembling the phasic IP_3-mediated contractions by phenylephrine in the absence of extracellular Ca^{2+}.

Because phasic phenylephrine-induced contractions in the absence of external Ca^{2+} are not sensitive to stimulation of endothelial cells with acetylcholine (Leloup et al., 2015a), it may be assumed that Ca^{2+} influx in the endothelial cells is a prerequisite to activate eNOS and induce NO release. The occurrence of phasic contractions by phenylephrine in the presence of levcromakalim and GSK-7975A and, hence, absence of Ca^{2+} influx via VGCC and NSCC, suggests that 1) influx of Ca^{2+} in the endothelial cells is not sensitive to levcromakalim and GSK-7975A; 2) even in this situation contractile SR Ca^{2+} stores are refilled as long as external Ca^{2+} is present and 3) because these phasic contractions increased with time after mounting the segments, parallel with the estimated amount of basal NO release from the endothelial cells, basal NO release controls contractile store refilling in the aortic VSMCs. It should be mentioned that contractile store-refilling characteristics may differ between smooth muscle cells of different origin, because in mouse urethral smooth muscle cells, it was shown that spontaneous or PE-evoked Ca^{2+} signalling and contractions were inhibited with GSK-7975A (1-10 µM), but not with L- or T-type Ca^{2+} channel inhibitors. We also tested the non-contractile store-refilling in aortic segments and found that 10 µM GSK-7975A completely inhibited non-contractile Ca^{2+} influx in cyclopiazonic acid-mediated store emptying (Fig. 7), confirming the higher affinitiy of GSK-9795A for non-contractile Ca^{2+} influx comparable with the action of Synta-66.

4.3. Indirect effects of GSK-7975A on VGCC.

By inhibiting basal NO release, L-NAME causes depolarization of the membrane potential of VSMC (Bratz et al., 2002; Fan et al., 2007; Krippeit-Drews et al., 1992), removes the NO mediated inhibition of NSCC Ca^{2+} influx ((Cohen et al., 1999; Trepakova et al., 1999), inactivates Ca^{2+} activated K^+ currents (BK_{Ca}) (Bolotina et al., 1994) and causes window VGCC Ca^{2+} influx at the resting potential of the VSMC (Fransen et al., 2012a). If GSK-7975A has a higher affinity for NSCC than for VGCC mediated contractions, it is expected that a concentration of GSK-7975A (10 µM), which causes complete inhibition of NSCC and partial inhibition of VGCC, has a repolarizing effect on the membrane potential of VSMC depolarized

with L-NAME. GSK-7975A caused significant shifts of the K^+-contraction curves in the same direction as exogenous NO or levcromakalim, but in the opposite direction of L-NAME (Fig. 4B, C and D) (Fransen et al., 2012a; Fransen et al., 2012b).The shifts amounted 3.4 mM K^+ in the absence of L-NAME and 5.1 mM K^+ in the presence of L-NAME. Hence, the shifts induced by GSK-7975A were dependent on the presence of basal NO release, because there is less GSK-7975A-sensitive NSCC depolarizing activity in the presence of basal NO release.

4.4. GSK-7975A and NO release.

When aortic segments are mounted in isometric conditions, we have described that, during the course of an experiment, basal NO efficacy decreases with time after mounting (Leloup et al., 2017; van Langen et al., 2012). This is also evident in the present study as an increase of the phenylephrine-induced phasic and tonic contraction with time after mounting (Fig. 5). If GSK-7975A, similarly to basal NO release, causes repolarization of the membrane potential of VSMC as suggested by the shift of the K concentration-contraction curves, GSK-7975A may have the capacity to compensate for an important function of NO in case of endothelial dysfunction or inhibition of NO release by L-NAME. Indeed, in the presence of 10 µM GSK-7975A, the phenylephrine-induced contraction was almost completely inhibited 1h after segment mounting, whereas after 6 h, phenylephrine-induced contractions were still significantly inhibited, but with high variability between the mice. Thereby, it was observed that the lowest contractions at 1h, which were most inhibited by basal NO release were the contractions, which were also most inhibited at 6h. Hence, these contractions displayed minor time-dependency of basal NO release degradation. On the other hand, the highest contractions by phenylephrine at 1h, which were only minorly inhibited by basal NO release were the contractions, which were almost not inhibited with GSK-7975A at 6h (Fig. 6H). This means that the difference between contractions in control and in the presence of GSK-7975A is the NSCC-sensitive contraction. This contraction is more or less the same at 1 h or at 6 h, suggesting that the contraction mediated by VGCC Ca^{2+} influx is time-dependently increased, parallel with the decrease in basal NO release and concomitant depolarization.

After preventing phenylephrine-induced VGCC mediated Ca^{2+} influx by incubating the segments with 1 µM levcromakalim, the time-dependent increase of the phenylephrine elicited

isometric contraction remained and L-NAME caused a significant increase of contraction, which was independent of the presence of 3.5µM diltiazem, hence NSCC-mediated.

Conclusions

To summarize, GSK-7975A inhibits contractile Ca^{2+} influx in mouse aortic segments with different sensitivity and about ten times higher affinity for NSCC mediated Ca^{2+} influx (IC_{50}: 1.2 µM) than for VGCC mediated Ca^{2+} influx (IC50: 12 µM). However, GSK-7975A did not affect contractile SR store refilling. Because of the shifts of the K^+ concentration-contraction curves, GSK-7975A has properties of a repolarizing agent, probably by the inhibition of NSCC, which could be compared with NO effects on VSMCs in the mouse aorta. The high affinity of GSK-7975A for non-contractile Ca^{2+} influx in mouse aortic segments was confirmed. Studies using GSK-7975A to inhibit non-contractile SR store related processes such as these related with the VSMC phenotype switch from contractile to proliferative/synthetic in pathophysiology, should always take into account the inhibitory action of the drug on ion channels mediating contraction of the VSMCs.

Figure legend

Fig. 1: Relaxing effect of GSK7975A. Cartoons above the curves illustrate which Ca^{2+} influx channels and Ca^{2+} release mechanisms are involved to elicit contraction in the different experimental conditions (A-C, EC: endothelial cell, VSMC, vascular smooth muscle cell, Vm: membrane potential, SR: sarcoplasmic reticulum, SERCA: sarcoplasmic reticulum calcium pump, eNOS: endothelial NO synthase, VGCC: voltage-gated Ca2 channel, NSCC: non-selective cation channel, red arrow: inhibition). (D-F) Representative examples of the inhibition induced by cumulative concentrations of GSK-7975A (*: 0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 µM) on pre-contractions elicited by depolarization with 50 mM K^+ (D) or 2 µM phenylephrine (E, F) in the absence (control) and presence of 300 µM L-NAME (+LNAME). Phenylephrine was applied in the presence (E) or absence (F) of extracellular Ca^{2+}. In C, where phasic and tonic contraction are separated, phenylephrine elicited a phasic IP_3-mediated contraction (the transient contraction at the beginning of the trace) and a tonic contraction after re-addition of external Ca^{2+} (arrow). (G-I) show the respective concentration-inhibition curves (for I only for the tonic

contractions by PE) in the absence (open symbols) and presence (closed symbols) of 300 µM L-NAME to block basal NO release (n=3).

Fig. 2. Comparison of the relaxing effect of GSK7975A in different experimental conditions. The cartoon (A) shows that cyclopiazonic acid (CPA, blue) and levcromakalim (green) favour the relative contribution of NSCC over VGCC to the contraction produced by phenylephrine. Absolute (B) and relative (C) GSK-7975A (GSK) concentration-inhibition curves (n=5) for pre-contractions by 2 µM phenylephrine (+300 µM L-NAME) elicited in the absence (PE, black) and presence of 10 µM cyclopiazonic acid (PE + CPA, blue) or 1 µM levcromakalim (PE + Lev, green). For the relative curves, 100% contraction corresponded to the contraction in the absence of GSK-7975A. (D) IC_{50} values summary for inhibition by GSK-7975A of pre-contractions elicited by 50 mM K^+ (K50), 124 mM K^+ (K124), α_1-adrenoceptor stimulation with 2 µM phenylephrine alone or in the presence of 10 µM cyclopiazonic acid or 1 µM levcromakalim. White circles refer to log(IC_{50}) values in the absence of L-NAME. The presence of 300 µM L-NAME is indicated by the horizontal bar. *, **, ***: P<0.05, 0.01, 0.001 K versus PE, PE + Lev or PE + CPA versus PE (n=5)

Fig. 3: Re-filling of contractile phenylephrine-sensitive Ca^{2+} stores is GSK-7975A independent. A: Protocol used to study re-filling of phenylephrine-sensitive contractile SR Ca^{2+} stores. Re-filling of phenylephrine-sensitive contractile SR Ca^{2+} stores was studied in the absence and presence of GSK-7975A or diltiazem (arrow). Phasic contraction of the first phenylephrine application (PE1) was compared with the phasic contraction of the second phenylephrine application (PE2). B: Area under the curve (AUC) of the different phasic contractions by 2 µM phenylephrine in the absence of extracellular Ca^{2+}. Phasic ontractions are shown in C, F, I for PE1 and D, G, J for PE2. AUC was measured over a period of 75 seconds for the first (PE1) and second (PE2) phasic contraction in control (Co, open circles) and in the presence of 10 µM GSK (GSK, red) or 3.5 µM diltiazem (dil, purple). During this period, force development is mainly due to the phenylephrine-induced phasic contraction. E, H and K: Tonic contractions in the absence (+Ca (before test), open cicles) and presence of 10 µM GSK-7975A (red) and 3.5 µM diltiazem (purple) (+Ca (test), full circles). *, **, ***: P<0.05, 0.01, 0.001 (n=5). All experiments were performed in the presence of 300 µM L-NAME.

Fig. 4: GSK-7975A has properties of a membrane potential repolarizing agent. A: Increases of extracellular K^+ causes depolarization of membrane potential (V_m) and activation of VGCC. GSK-7579A may repolarize V_m and cause delayed activation of VGCC when K^+ is increased. Influence of 10 µM GSK-7975A on window contractions induced by depolarization of aortic segments (n=4 and 5) with elevated external K^+. Fig. B and C display absolute, respectively relative contractions in the absence and presence of LNAME to block NO production (squares) and in the absence and presence of GSK 79075A (red). The EC_{50} of K^+ in the four conditions is shown in Fig. D. *, **, ***: P<0.05, 0.01, 0.001 GSK-7975A versus control; ###: L-NAME versus without L-NAME.

Fig. 5: GSK-7975A affects the spontaneous decrease of mounting time-dependent basal NO release. Cartoons show the intracellular events mediating contractile effects of phenylephrine while basal NO is decreasing with time after mounting the segments in the absence (A) and presence (B) of levcromaklim (low VGCC Ca^{2+} influx) and GSK-7975A (low NSCC Ca^{2+} influx). Phenylephrine (2 µM)-induced contractions at different time intervals (time after mounting in hours indicated as a number from 1 up to 6 after the force traces in panel C) after mounting the aortic segments. Phenylephrine-induced isometric contractions in the absence (control, C) and presence of 10 µM GSK-7975A (D), 1 µM levcromakalim (E) and the combination of GSK-7975A with levcromakalim (GSK + Lev, F) at 1 up to 6h in hourly intervals (different colors) after mounting the segments. G displays the time-dependent increase of the contraction in the different conditions with the full lines representing the exponential fit of the data points. Circles: control (white), levcromakalim (green), GSK-7975A (red), triangles: combination of levcromaklim and GSK-7975A. *, **, ***: P<0.05, 0.01, 0.001 time point versus 1h, two way Anova with Dunnett's multiple comparison test. H shows the scatter plot for the steady-state contractions by 2 µM phenylephrine at 200 s for contractions measured at 6h. ***: P<0.001 versus Co, one way Anova with Dunnett's multiple comparison test,

Fig. 6. GSK-7975A and VGCC/NSCC contribution to phenylephrine-induced contractions. A: Cartoons display the intracellular events mediating contractile effects of phenylephrine in the different conditons, with L-NAME inhibiting basal NO release and promoting depolarization (upper), GSK-7975A inhibiting NSCC channels (middle) and levcromakalim promoting repolarization and preventing the activation of VGCC (bottom). To control for remaining

contribution of VGCC after the addition of L-NAME in the different conditions, 35 µM diltiazem was added. B: Phenylephrine (2 µM) induced tonic contraction amplitudes measured at 6h after mounting the mouse aortic segments in the different experimental conditions of Fig. 5 (circles) and following subsequent addition of 300 µM L-NAME (squares) and 35 µM diltiazem (half squares). Only in three of the seven experiments with GK-7975A, LNAME and diltiazem were added. **, ***: P<0.01, 0.001 control versus L-NAME, #, ###: P<0.05, 0.001 diltiazem versus L-NAME, one-way anova with Dunnett's multiple comparison test

Fig. 7. GSK-7975A has high affinity for non-contractile SR Ca^{2+} stores. Intracellular Ca^{2+} (A) and concomitant tension (B) upon addition of 10 µM cyclopiazonic acid to 0Ca (+CPA), re-addition of 3.5 mM Ca^{2+} (+Ca), inhibition of VGCC Ca^{2+} influx with 35 µM diltiazem (+dil) and inhibition of NSCC Ca^{2+} influx with 10 µM GSK-7975A (+GSK). The dashed lines indicate the mean Ca^{2+} and tension response after depolarization of the VSMC with 50 mM K^+ (n=5).In C, the cartoon displays the expected intracellular events in this experiment. CPA mainly causes release from the non-contractile SR Ca^{2+} store by inhibiting the re-uptake to this store. The Ca^{2+} influx pathway after emptying the SR store is mainly via NSCC and not via VGCC. Numbers in C refer to the main Ca^{2+} and tension events as shown in B. D shows mean Ca^{2+} signals and tension values (±S.E.M.) for 5 experiments. *,**: P<0.05, 0.01 versus KR, one-way anova with Dunnett's multiple comparison test

Funding

This work was supported by the University of Antwerp (GOA-BOF, grant 33931 to SDM). DDM and IC were fellows of the FWO-Flanders.

Author contributions:

Paul Fransen: Conceptualization, Methodology, Data curation, Formal analysis, Supervision, Writing- Original draft preparation, Writing- Reviewing and Editing. **Sofie De Moudt, Dorien De Munck and Isabelle Coornaert:** Formal analysis, Writing- Reviewing and Editing

Funding: This work was supported by the Fund for scientific Research (FWO)-Flanders (grant N° G.0412.16N), and the University of Antwerp (BOF). Dorien De Munck and Isabelle Coornaert were fellows of the FWO-Flanders. Sofie De Moudt and this experimental work were supported by the University of Antwerp (GOA-BOF, grant 33931).

Acknowledgments: With special thanks to Mr. Tim Coenen, PharmD, for excellent help with the experiments.

REFERENCES

Avila-Medina, J., Mayoral-Gonzalez, I., Dominguez-Rodriguez, A., Gallardo-Castillo, I., Ribas, J., Ordonez, A., Rosado, J.A., Smani, T., 2018. The Complex Role of Store Operated Calcium Entry Pathways and Related Proteins in the Function of Cardiac, Skeletal and Vascular Smooth Muscle Cells. Front Physiol 9, 257.

Berra-Romani, R., Mazzocco-Spezzia, A., Pulina, M.V., Golovina, V.A., 2008. Ca^{2+} handling is altered when arterial myocytes progress from a contractile to a proliferative phenotype in culture. Am.J.Physiol Cell Physiol 295, C779-C790.

Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J., Cohen, R.A., 1994. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature 368, 850-853.

Bratz, I.N., Falcon, R., Partridge, L.D., Kanagy, N.L., 2002. Vascular smooth muscle cell membrane depolarization after NOS inhibition hypertension. Am.J.Physiol Heart Circ.Physiol 282, H1648-H1655.

Cahalan, M.D., Zhang, S.L., Yeromin, A.V., Ohlsen, K., Roos, J., Stauderman, K.A., 2007. Molecular basis of the CRAC channel. Cell Calcium 42, 133-144.

Calderon-Sanchez, E.M., Avila-Medina, J., Callejo-Garcia, P., Fernandez-Velasco, M., Ordonez, A., Smani, T., 2020. Role of Orai1 and L-type CaV1.2 channels in Endothelin-1 mediated coronary contraction under ischemia and reperfusion. Cell Calcium 86, 102157.

Cohen, R.A., Weisbrod, R.M., Gericke, M., Yaghoubi, M., Bierl, C., Bolotina, V.M., 1999. Mechanism of nitric oxide-induced vasodilatation: refilling of intracellular stores by sarcoplasmic reticulum Ca^{2+} ATPase and inhibition of store-operated Ca^{2+} influx. Circ.Res. 84, 210-219.

De Moudt, S., Leloup, A., Van Hove, C., De Meyer, G., Fransen, P., 2017. Isometric Stretch Alters Vascular Reactivity of Mouse Aortic Segments. Front Physiol 8, 157.

Derler, I., Plenk, P., Fahrner, M., Muik, M., Jardin, I., Schindl, R., Gruber, H.J., Groschner, K., Romanin, C., 2013a. The extended transmembrane Orai1 N-terminal (ETON) region combines binding interface and gate for Orai1 activation by STIM1. J Biol Chem 288, 29025-29034.

Derler, I., Schindl, R., Fritsch, R., Heftberger, P., Riedl, M.C., Begg, M., House, D., Romanin, C., 2013b. The action of selective CRAC channel blockers is affected by the Orai pore geometry. Cell Calcium 53, 139-151.

Drumm, B.T., Rembetski, B.E., Cobine, C.A., Baker, S.A., Sergeant, G.P., Hollywood, M.A., Thornbury, K.D., Sanders, K.M., 2018. Ca(2+) signalling in mouse urethral smooth muscle in situ: role of Ca(2+) stores and Ca(2+) influx mechanisms. J Physiol 596, 1433-1466.

Fan, P., Li, L., Liu, Z.J., Si, J.Q., Zhang, Z.Q., Zhao, L., Ma, K.T., 2007. ACh-evoked membrane hyperpolarization in smooth muscle cells of rat vas deferens in vitro: involvement of K(+) channels and NO. Sheng Li Xue Bao 59, 331-338.
Fransen, P., Van Hove, C.E., J., v.L., Schrijvers, D.M., Martinet, W., De Meyer, G.R., Bult, H., 2012a. Contribution of transient and sustained calcium influx, and sensitization to depolarization-induced contractions of the intact mouse aorta. BMC.Physiol 12:9, doi:10.1186/1472-6793-1112-1189.
Fransen, P., Van Hove, C.E., Leloup, A.J., Martinet, W., De Meyer, G.R., Lemmens, K., Bult, H., Schrijvers, D.M., 2015. Dissecting out the Complex Ca2+-Mediated Phenylephrine-Induced Contractions of Mouse Aortic Segments. PLoS.One. 10, e0121634.
Fransen, P., Van Hove, C.E., van Langen, J., Bult, H., 2012b. Contraction by Ca^{2+} influx via the L-type Ca^{2+} channel voltage window in mouse aortic segments is modulated by nitric oxide. In: Sugi H (editor).Current basic and pathological approaches to the function of muscle cells and tissues-From molecules to Humans.Rijeka, Croatia: Intech (www.intech.com), 69-92.
Krippeit-Drews, P., Morel, N., Godfraind, T., 1992. Effect of nitric oxide on membrane potential and contraction of rat aorta. J Cardiovasc Pharmacol 20 Suppl 12, S72-75.
Kudryavtseva, O., Aalkjaer, C., Matchkov, V.V., 2013. Vascular smooth muscle cell phenotype is defined by Ca2+-dependent transcription factors. FEBS J 280, 5488-5499.
Leloup, A., De Moudt, S., Van Hove, C., Fransen, P., 2017. Cyclic Stretch Alters Vascular Reactivity of Mouse Aortic Segments. Front Physiol 8, 858.
Leloup, A.J., Van Hove, C.E., De Meyer, G.R., Schrijvers, D.M., Fransen, P., 2015a. Basal activity of voltage-gated Ca(2+) channels controls the IP3-mediated contraction by alpha(1)-adrenoceptor stimulation of mouse aorta segments. Eur J Pharmacol 760, 163-171.
Leloup, A.J., Van Hove, C.E., Heykers, A., Schrijvers, D.M., De Meyer, G.R., Fransen, P., 2015b. Elastic and Muscular Arteries Differ in Structure, Basal NO Production and Voltage-Gated Ca(2+)-Channels. Front Physiol 6, 375.
Li, J., McKeown, L., Ojelabi, O., Stacey, M., Foster, R., O'Regan, D., Porter, K.E., Beech, D.J., 2011. Nanomolar potency and selectivity of a Ca(2)(+) release-activated Ca(2)(+) channel inhibitor against store-operated Ca(2)(+) entry and migration of vascular smooth muscle cells. Br J Pharmacol 164, 382-393.
Ruhle, B., Trebak, M., 2013. Emerging roles for native Orai Ca2+ channels in cardiovascular disease. Curr Top Membr 71, 209-235.
Trepakova, E.S., Cohen, R.A., Bolotina, V.M., 1999. Nitric oxide inhibits capacitative cation influx in human platelets by promoting sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase-dependent refilling of Ca^{2+} stores. Circ.Res. 84, 201-209.
Van Hove, C.E., Van der Donckt, C., Herman, A.G., Bult, H., Fransen, P., 2009. Vasodilator efficacy of nitric oxide depends on mechanisms of intracellular calcium mobilization in mouse aortic smooth muscle cells. Br.J.Pharmacol. 158, 920-930.
van Langen, J., Fransen, P., Van Hove, C.E., Schrijvers, D.M., Martinet, W., De Meyer, G.R., Bult, H., 2012. Selective loss of basal but not receptor-stimulated relaxation by endothelial nitric oxide synthase after isolation of the mouse aorta. Eur.J.Pharmacol. 696, 111-119.
Zhang, X., Xin, P., Yoast, R.E., Emrich, S.M., Johnson, M.T., Pathak, T., Benson, J.C., Azimi, I., Gill, D.L., Monteith, G.R., Trebak, M., 2020. Distinct pharmacological profiles of ORAI1, ORAI2, and ORAI3 channels. Cell Calcium 91, 102281.

Figures:

FIGURE 1

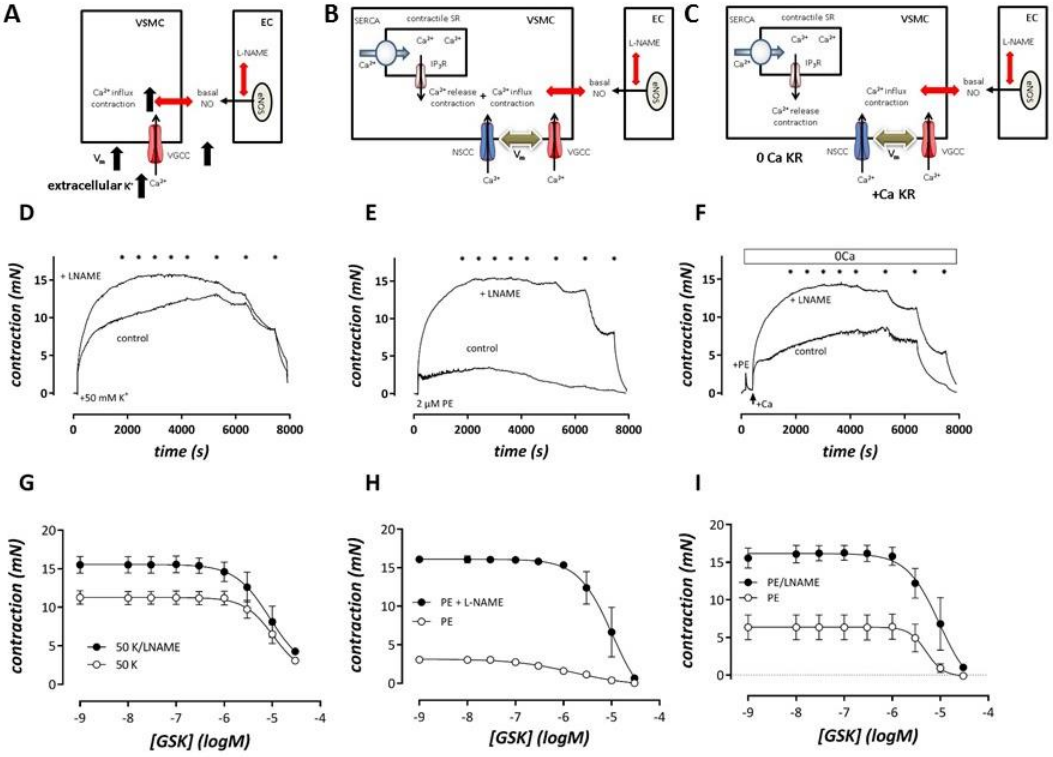


FIGURE 2

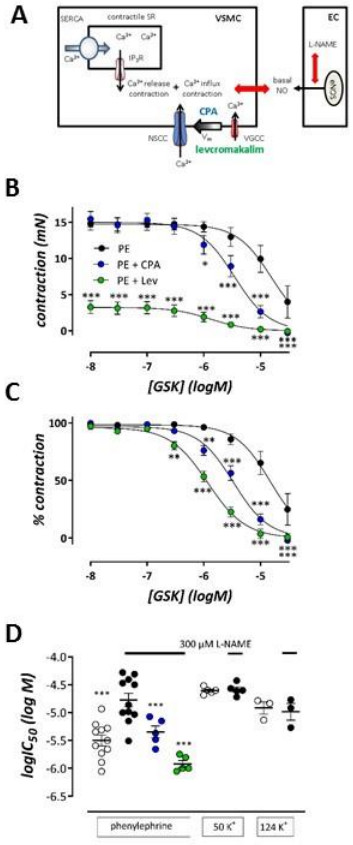


FIGURE 3

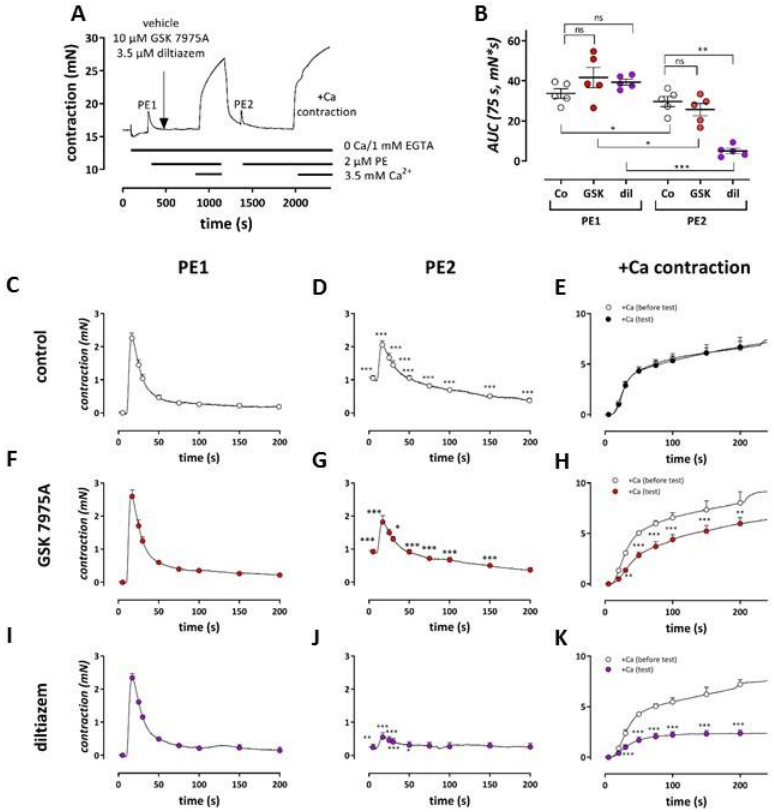


FIGURE 4

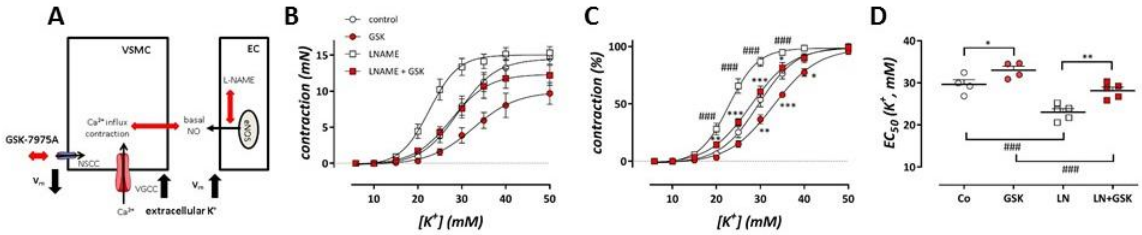


FIGURE 5

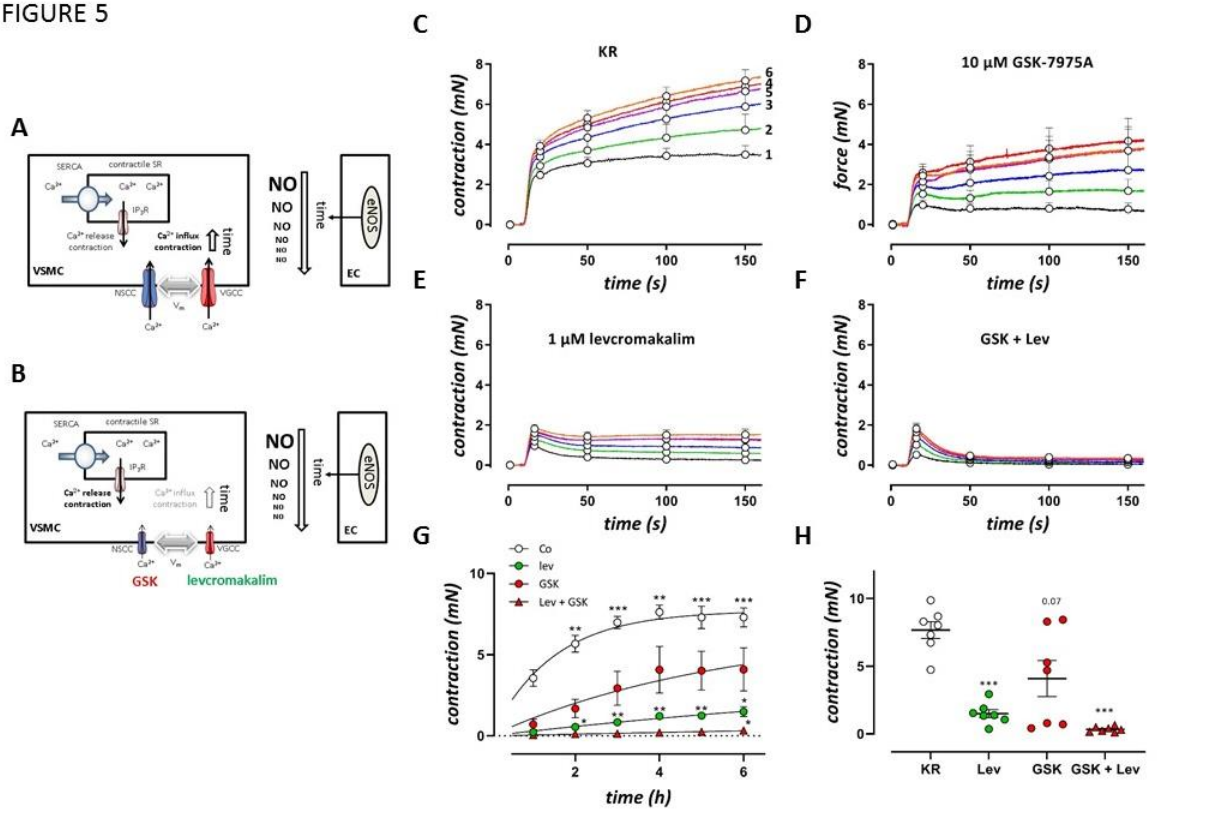


FIGURE 6

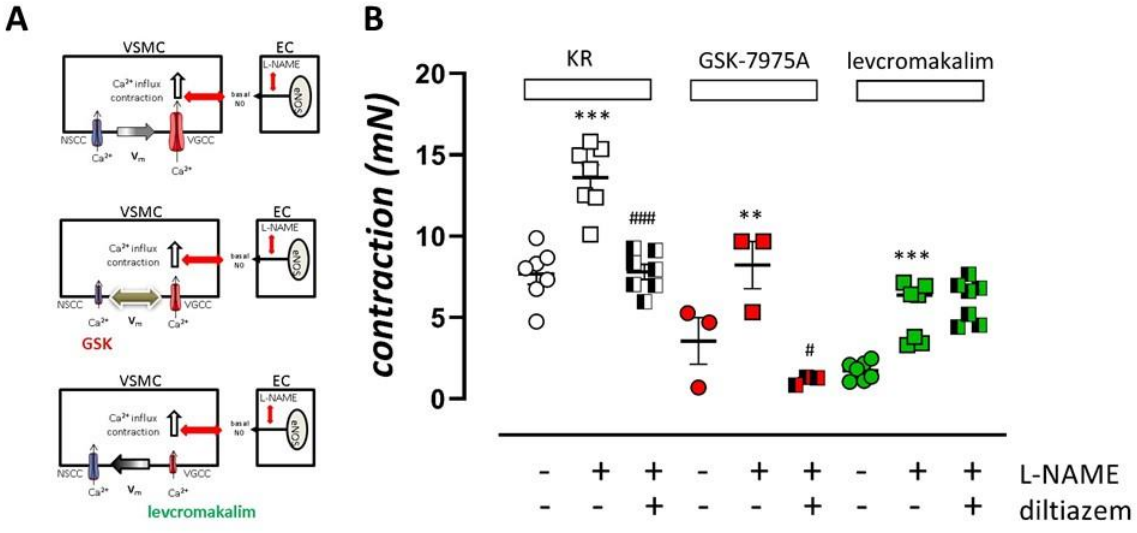
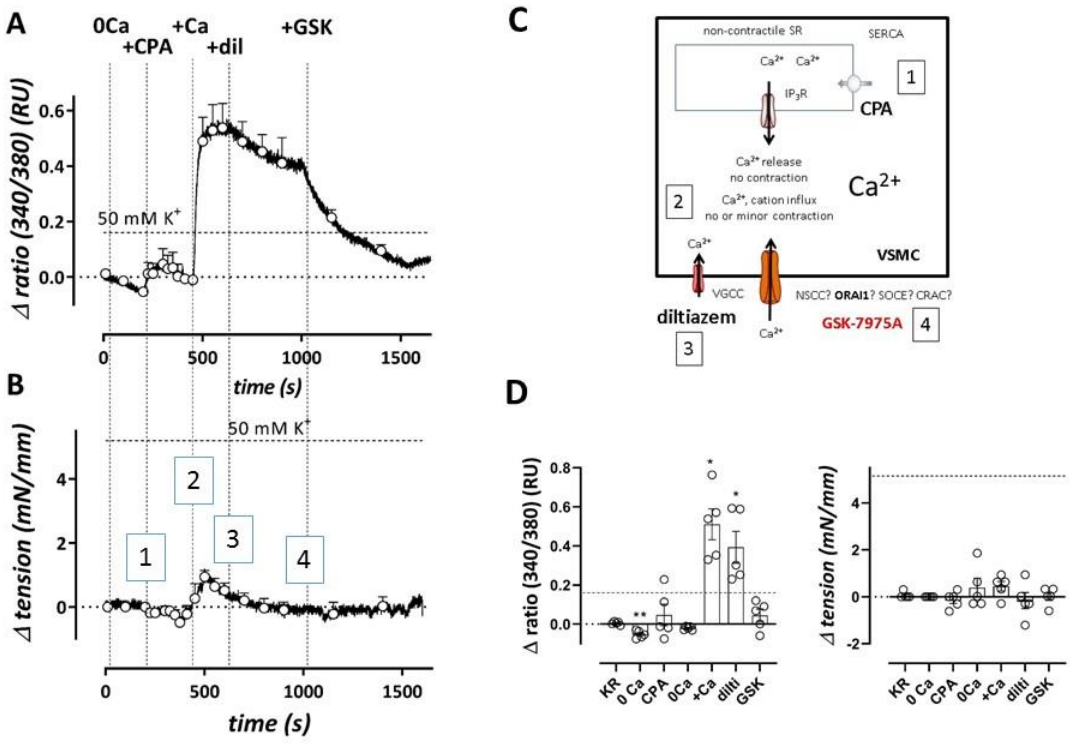


FIGURE 7


A
0Ca
+Ca
+GSK
+CPA
+dil
Δ ratio (340/380) (RU)
50 mM K+
time (s)
B
Δ tension (mN/mm)
50 mM K+
1
2
3
4
time (s)
C
non-contractile SR
SERCA
Ca2+
IP3R
CPA
Ca2+ release
no contraction
Ca2+, cation influx
no or minor contraction
VSMC
VGCC
NSCC? ORAI1? SOCE? CRAC?
diltiazem
GSK-7975A
D
Δ ratio (340/380) (RU)
Δ tension (mN/mm)
KR
0 Ca
CPA
0Ca
+Ca
dilti
GSK