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Characterization of the CGRP receptor antagonist telcagepant (MK-0974) in human isolated coronary arteries

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Running title: Effect of telcagepant in human coronary arteries

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Non-standard Abbreviations and Acronyms

CGRP	calcitonin gene-related peptide
RAMP1	receptor activity modifying protein 1
CLR	calcitonin-like receptor
cAMP	cyclic adenosine monophosphate
LIMA	left internal mammary artery

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$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
$5\text{-}HT_{1B/1D}$	5-hydroxytryptamine$_{1B/1D}$
IBMX	isobutylmethylxanthine
PBST	phosphate buffered-saline (PBS) containing 0.25% Triton X-100
BSA	bovine serum albumin

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Abstract

The sensory neuropeptide calcitonin gene-related peptide (CGRP) plays a role in primary headaches and CGRP receptor antagonists are effective in migraine treatment. CGRP is a potent vasodilator raising the possibility that antagonism of its receptor could have cardiovascular effects. We therefore investigated the effects of the anti-migraine CGRP receptor antagonist telcagepant (MK-0974) on human isolated coronary arteries. Arteries with different internal diameters (ID) were studied to assess the potential for differential effects across the coronary vascular bed. The concentration-dependent relaxation responses to human αCGRP were greater in distal (ID: 600-1000 μm, E_{max}=83±7%) than proximal coronary arteries (ID: 2-3 mm, E_{max}=23±9%), coronary arteries from explanted hearts (ID 3-5 mm, E_{max}=11±3%) and coronary arterioles (ID: 200-300 μm, E_{max}=15±7%). Telcagepant alone did not induce contraction or relaxation of these coronary blood vessels. Pre-treatment with telcagepant (10 nM to 1 μM) antagonized αCGRP-induced relaxation competitively in distal coronary arteries (pA_2 =8.43±0.24), proximal coronary arteries and coronary arterioles (telcagepant 1 μM giving pK_B =7.89±0.13 and 7.78±0.16 respectively). αCGRP significantly increased cAMP levels in distal, but not proximal coronary arteries and this was abolished by pretreatment with telcagepant. Immunohistochemistry revealed the expression and co-localization of the CGRP1 receptor elements calcitonin like receptor (CLR) and receptor activity modifying protein 1 (RAMP1) in the smooth muscle cells in the media layer of human coronary arteries. These findings in vitro support the cardiovascular safety of CGRP receptor antagonists and suggest that telcagepant is unlikely to induce coronary side effects under normal cardiovascular conditions.

Introduction

Migraine is thought to be a neurovascular disorder although its pathophysiology remains elusive. In contrast the physiology and pharmacology of migraine pain is undoubtedly associated with activation of the trigeminovascular sensory nervous system (Silberstein, 2004; Arulmani et al., 2006). The trigeminovascular system contains calcitonin gene-related peptide (CGRP)-positive trigeminal sensory nerves that innervate cerebral and meningeal blood vessels and with their central synapses mediate pain signal transmission to central second order sensory neurons within the brainstem trigeminal nucleus caudalis. Activation of the trigeminal nerves during migraine pain has been shown to be associated with the release of CGRP (reviewed in Edvinsson and Linde, 2010) and its importance in the pathophysiology of migraine pain has been confirmed pharmacologically by the clinical anti-migraine efficacy of the CGRP receptor antagonist olcegepant (BIBN4096BS) given intravenously (Olesen et al., 2004).

The human coronary circulation is densely innervated with CGRP-positive fibers (Gulbenkian et al., 1993) raising the possibility that CGRP receptor antagonists could affect coronary vascular tone. In the current experiments, we have investigated *in vitro* the potential coronary vascular safety profile of telcagepant (MK-0974) a novel orally bioavailable anti-migraine CGRP receptor antagonist (Paone et al., 2007; Ho et al., 2008; Connor et al., 2009) that is currently in late phase clinical trials. The studies investigated the anatomical localization of CGRP1 receptors in coronary vessels using immunohistochemistry and compared the coronary vascular pharmacology of telcagepant with the vasoconstrictor anti-migraine 5-$HT_{1B/1D}$ receptor agonist zolmitriptan in human coronary arteries of different diameter to assess its potential cardiovascular safety profile.

Materials and Methods

Human isolated arteries

The right proximal (internal diameter 2-3 mm) and distal (internal diameter 600-1000 μm) coronary arteries were obtained within 24 hours after death from heart beating organ donors (8 male, 11 female; age 19-64 years). The donor hearts were provided by the Rotterdam Heart Valve Bank through the Bio Implant Services Foundation / Eurotransplant Foundation (Leiden, The Netherlands) after removal of the aortic and pulmonary valves for homograft valve transplantation. In Sweden, 1) the left internal mammary artery (LIMA, internal diameter 3-5 mm) was removed in conjunction with coronary artery by-pass surgery (5 male, 1 female; age 62-78 years). 2) Epicardial arteries of somewhat larger diameter (internal diameter 3-5 mm) were removed from 2 explanted hearts in conjunction with heart transplantation (1 male, 1 female, age 56 and 67 years), and 3 coronary arterioles of small diameter (about 300 μm) were removed during valvular surgery (5 male, 4 female; age 67-84 years). All vessels were placed in buffer solution aerated with 5% CO_2 in O_2 (carbogen) for transfer to the participating laboratories. The buffer composition (mM) in the Netherlands was NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 8.3; pH 7.4 and in Sweden NaCl, 119; KCl, 4.7; $CaCl_2$, 1.5; $MgSO_4$, 1.17; $NaHCO_3$, 25; KH_2PO_4, 1.18; EDTA, 0.027; glucose, 5.5, pH 7.4 The Swedish study had the individual patients' approval and was sanctioned by the Lund University Ethics Committee (LU99) and the Human Ethics committee at Erasmus Medical Center, Rotterdam, approved the Dutch experiments.

Functional experiments

For *in vitro* pharmacological experiments the arteries were each cut into cylindrical segments of one to four mm length. Each segment was mounted on two metal prongs, one of which was connected to a force displacement transducer and attached to a computer, and the other to a

displacement device. The mounted specimens were immersed in temperature-controlled tissue baths (37°C) containing the buffer solution continuously gassed with carbogen, and the artery segments were allowed to equilibrate for approximately 30 min. Resting tone was 4 mN for LIMA, coronary arteries obtained from explanted hearts and coronary arterioles, and about 15 mN for the proximal coronary artery segments obtained from heart beating organ donors. Distal segments obtained from the heart beating organ donors were stretched to a tension normalized to 90% of the diameter when transmural pressure equals 100 mmHg (Mulvany and Halpern, 1977).

For the experiments in Sweden, after a 30-min equilibration period, the contractile capacity of each vessel segment was examined by exposure to a potassium-rich (60 mM) buffer solution that had the same composition as the standard buffer solution, except that the NaCl was exchanged for an equimolar concentration of KCl. For the experiments in the Netherlands, vessel segments were exposed to 30 mM KCl once (distal segments) or twice (proximal segments). The functional integrity of the endothelium was verified by observing relaxation to substance P (1-10 nM) after pre-contraction with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 1 μM, proximal coronary artery segments (MaassenVanDenBrink et al., 1998)) or the thromboxane A_2 analogue U46619 (10 nM, distal segments (Batenburg et al., 2006). After washout, the maximum contractile response of the tissue to 60 mM KCl was determined. The relaxant (vasodilator) effect of human αCGRP (h-αCGRP) was examined by cumulative application of increasing concentrations of the peptide in the absence or presence of various concentrations of the antagonist telcagepant (MK-0974); preliminary data have been presented on autopsy large coronary arteries (Lynch et al, 2010). The Dutch experiments used both human and rat αCGRP and the data was pooled for analysis as they were equipotent. Each segment was pre-contracted with 1 μM U46619 (Swedish experiments) or

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30 mM KCl (Dutch experiments) before αCGRP was added. Pilot experiments (data not shown) showed that these different pre-contraction protocols did not affect the vasorelaxant (vasodilator) response to CGRP. In the Dutch experiments, a matched pair's protocol was used where two vessel segments from the same artery were exposed to a single cumulative concentration-effect curve with one segment acting as control (no antagonist present), and the other to assess the agonist response following equilibration (30 min) with various concentrations of the antagonist. In the Swedish experiments, in view of the scarcity of tissue, cumulative concentration-effect curves were performed in the absence or presence of one or two concentrations of the antagonist in the same segments. The first curve acted as control (no antagonist present). After proper washout and return to baseline, the next curve was then performed in the presence of the antagonist (10 nM, 100 nM or 1 μM).

In a separate set of experiments, contractions to the 5-hydroxytryptamine$_{1B/1D}$ (5-HT$_{1B/1D}$) receptor agonist, zolmitriptan were compared to contractile effects of telcagepant and the relaxations to αCGRP.

cAMP measurements

Human proximal and distal coronary artery segments obtained from heart beating organ donors in The Netherlands were incubated in a medium containing isobutylmethylxanthine (IBMX, 0.5 mM) for 30 min in the absence or presence of telcagepant (1 μM, separate segments as those used for myograph studies). The arterial segments were exposed to h-αCGRP (10 μM) or forskolin (10 μM) for 5 min and then snap frozen. Forskolin, which increases intracellular cAMP concentrations by activating adenylyl cyclase, was used to assess the specificity of telcagepant towards CGRP-mediated increases in cAMP

concentrations. The samples were stored at -80°C until cAMP assay using the ELISA kit and manual (R&D Systems Europe Ltd., Abingdon, U.K.).

Compounds

The following materials were used in the *in vitro* experiments: human and rat αCGRP (NeoMPS S.A., Strasbourg, France, and Sigma, U.S.A., for the Dutch and Swedish experiments, respectively), Prostaglandin $F_{2\alpha}$ (tris salt) and U46619 (9, 11-dideoxy-11α, 9α-epoxy-methano-prostagladin $F_{2\alpha}$, Sigma, U.S.A.). Zolmitriptan (from nasal spray, AstraZeneca, U.K. or AK Scientific, U.S.A., for the Swedish and Dutch experiments, respectively) was dissolved in saline. Telcagepant (MK-0974) was synthesized and supplied by the Medicinal Chemistry department, Merck, West Point PA, U.S.A. αCGRP and U46619 were dissolved in water and stored as aliquots at -20°C. Telcagepant was dissolved in dimethylsulphoxide (DMSO) and stored as aliquots at -20°C. The compounds were diluted in saline for use in the experiments.

Analysis of data

The vasodilator response was expressed relative to the contraction evoked by U46619 or KCl, respectively (= 100%). For each segment the maximum vasodilator effect (E_{max}) was calculated. The concentration response curves for all agonists were analysed using nonlinear regression analysis and the potency of agonists was expressed as pEC_{50} (i.e., negative logarithm of the molar concentration of agonist inducing half maximum response) using Graph Pad Prism 4.0 (Graph Pad Software Inc., San Diego, CA, U.S.A.). The blocking potency of the antagonist was estimated by calculating EC_{50} ratios and plotting a Schild plot (Arunlakshana and Schild, 1959) using linear regression to get the slope value. In proximal coronary arteries and coronary arterioles, only one concentration of telcagepant was studied,

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in these cases "apparent pK_B" values were calculated, constraining the slope to unity. Since it was not feasible to use agonist concentrations higher than 3 μM, concentration response curves in the presence of higher concentrations of antagonist did not always reach a plateau. In these cases, the concentration response curves were extrapolated, considering the maximal response in the absence of antagonist as E_{max}. Data are expressed as mean values ± S.E.M. (standard error of mean) and 'n' refers to the number of patients from whom the vessels were collected. Statistically significant differences in pEC_{50} values were examined by Mann Whitney U-test, correlations were studied using the SPSS 15.0 statistical program (SPSS, Chicago, IL, U.S.A.). The potency of telcagepant can be compared across the various arterial preparations since each potency value is calculated in relation to its own control.

Immunohistochemistry

For immunofluorescence studies, the distal artery and arteriole segments were embedded in Tissue TEK (Gibco, Invitrogen A/S, Taastrup, Denmark), frozen at -80°C and subsequently sectioned into 10 μm thick slices. Cryostat sections were fixed for 10 minutes in ice-cold acetone (-20°C) and thereafter rehydrated in phosphate buffered-saline (PBS, pH 7.2) containing 0.25% Triton X-100 (PBST), for 3x5 minutes. The sections were then blocked for 1 h in blocking solution containing PBS and 5% normal donkey serum and then incubated overnight at 4°C with either of the following primary antibodies: rabbit anti RAMP1(1:50, Santa Cruz Biotechnology, CA, U.S.A., sc-11379), rabbit anti CLR (1:100, Alpha Diagnostic International, SA, U.S.A., CRLR-11A). The primary antibodies were diluted in PBST, 1% bovine serum albumin (BSA) and 3% normal donkey serum. On the second day sections were room-tempered and rinsed in PBST for 3x15 minutes. Sections were subsequently incubated with secondary antibody (1h, room temperature). The secondary antibody used was Cy™2 conjugated donkey anti rabbit (1:200, Jackson ImmunoResearch, West Grove, PA, U.S.A,

711-165-152) diluted in PBST and 1% BSA. The sections were washed subsequently with PBST and mounted with Crystal mounting medium (Sigma, St.Louis, MO, U.S.A.). To determine cellular localization of RAMP1 and CLR, double immunofluorescence was performed by addition of a mouse anti smooth muscle actin antibody (1:200, Santa Cruz, sc-53015). As secondary antibody Texas Red-conjugated donkey anti-mouse was used (1:200, Jackson ImmunoResearch, 715-076-150). To co-localize RAMP1 and CLR, an additional anti-goat CLR antibody, was used (1:50, Santa Cruz, sc-18007). As secondary antibody, Alexa Flour 488 donkey anti-goat was used for the double staining (1:400, Invitrogen, La Jolla, CA, U.S.A). Vectashield medium containing 4', 6-diamidino-2-phenylindole (DAPI) staining nuclei was used on some sections (Vectashield, Vector Laboratories Inc, Burlingame CA, U.S.A.).

Immunoreactivity was visualized with an Olympus Microscope (BX 60, Japan) at the appropriate wavelength. Adobe Photoshop CS3 was to visualise co-labelling by superimposing the digital images. Negative controls for all antibodies were made by excluding primary antibodies and in all cases resulted in no specific staining; only auto fluorescence in lamina elastica interna was seen (not shown). As controls, to evaluate the auto fluorescence in lamina elastica interna, preparations were made using only the primary antibodies.

Results

Functional studies to αCGRP in human isolated arteries

Pre-contracted proximal coronary arteries did not consistently relax to αCGRP in four out of eight experiments. The mean E_{max} to CGRP, including both responding and non-responding arteries, amounted to 23 ± 9% (n=8, Figure 1, upper left panel) of pre-contraction induced by 30 mM KCl. The mean pEC_{50} of the responding proximal coronary arteries was 7.2 ± 0.2. In coronary arteries obtained from 2 explanted hearts, that had somewhat larger diameter than the proximal coronary arteries from healthy heart beating donors, αCGRP induced a small relaxation after pre-contraction with U46619 (E_{max} of 11 ± 3% and pEC_{50} of 8.1 ± 0.1). In distal coronary arteries αCGRP induced consistent relaxant responses with an E_{max} of 83 ± 7% of pre-contraction induced by 30 mM KCl and a pEC_{50} of 9.1 ± 0.1 (n=6, Figure 1, middle left panel). The small human coronary arterioles obtained from patients undergoing valvular surgery relaxed upon administration of αCGRP in a consistent concentration-dependent manner with a smaller E_{max} of 15 ± 7% and a pEC_{50} of 8.0 ± 0.1 (Figure 1, lower left panel). There were no noticeable relaxant responses to αCGRP in segments of the LIMA (data not shown).

Effects of telcagepant in human isolated arteries

The CGRP receptor antagonist telcagepant, tested in concentrations up to 100 μM, did not show any vasomotor (contraction or relaxation) responses in any of the isolated vessel segments at basal tone (Figure 2). Pre-treatment with telcagepant at increasing concentrations (10 nM to 1 μM) caused concentration-dependent parallel shifts to the right of the concentration-effect curve to αCGRP without changing the maximum relaxant response in proximal (Figure 1, upper left panel) or distal coronary arteries (Figure 1, middle left panel)

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and small coronary arterioles from valvular surgery patients (Figure 1, lower left panel). The pA_2 value was 8.43±0.24 in distal coronary arteries; the slope of the Schild plot did not differ from unity (slope= 0.8±0.1, p = 0.07) (Figure 1, middle right panel). Although in the proximal coronary artery and the small coronary arterioles, αCGRP was less potent and less efficacious as a relaxant agent compared to that seen in distal coronary arteries, telcagepant was still an effective antagonist with a apparent pK_B of 7.78 ± 0.16 for the proximal coronary artery and 7.89 ± 0.13 for the coronary arterioles (pA_2 could not be calculated since only one concentration of telcagepant was studied) (Figure 1, upper and lower right panels).

Effect of zolmitriptan in human isolated arteries

In proximal and distal coronary arteries, zolmitriptan induced a concentration-dependent contraction, which was larger in distal (99 ± 44%, n = 4) than in proximal (13 ± 3%, n = 7) segments (Figure 2, upper panels). In the coronary arterioles, zolmitriptan induced a strong concentration-dependent contraction (155 ± 47%, n = 4, Figure 2, lower left panel). All these vessel segments also responded to αCGRP with relaxation; the E_{max} of zolmitriptan was unrelated to that of αCGRP (Pearson's correlation < 0.4 and p > 0.05, Figure 2, lower right panel).

Effect of αCGRP and telcagepant on cAMP levels

In proximal coronary arteries, αCGRP, either in the absence or presence of telcagepant, did not increase cAMP levels (Figure 3, upper left panel). In contrast, αCGRP increased cAMP levels in distal coronary arteries, which was abolished by pretreatment with telcagepant (Figure 3, upper right panel). Forskolin increased the cAMP levels in both proximal and distal coronary arteries; this increase was not inhibited by pretreatment with telcagepant (Figure 3,

lower panels). Telcagepant, in concentrations up to 1 μM, did not affect cAMP levels at baseline (data not shown).

Immunohistochemistry of human arteries

The distributions of RAMP1 and CLR in distal coronary arteries (Figure 4, upper panel) and coronary arterioles (Figure 4, lower panel) were studied by immunohistochemistry. Positive immunoreactivity for RAMP1 and CLR was observed in the smooth muscle layer (media layer) of coronary artery segments. The localization of CGRP receptor components in the smooth muscle layer was confirmed by double staining with an antibody specific for actin, which showed a clear co-localization. With the use of another CLR antibody, we could verify that the two receptor components RAMP1 and CLR co-localized, supporting the presence of functional CGRP1 receptor in these arteries (Figure 5). There were no obvious positive immunoreactions in the endothelium or in the lamina elastica interna; the latter is strongly auto-fluorescent, especially in the green wavelength.

Discussion

In the current studies, CGRP-induced concentration-dependent relaxations varied in magnitude in coronary arteries of different caliber and were independent of the endothelial quality of the vessel segments and the presence of coronary artery disease. Indeed, similar responses were observed in coronary arteries of similar diameter taken from explanted hearts, and healthy heart-beating donors (Gulbenkian et al., 1993; Hasbak et al., 2003; Gupta et al., 2006b). CGRP-induced relaxation was most pronounced in distal coronary arteries than in proximal coronary arteries, coronary arteries from explanted hearts and small coronary arterioles. Our immunohistochemical studies showed no profound difference in the immunoreactivity of CLR and RAMP1, the CGRP receptor components, between proximal and distal coronary arteries, suggesting that the differential dilator effects of CGRP are not due to differences in CGRP receptor density between arteries but may reflect differences in efficiency of intracellular receptor coupling. Interestingly, the EC_{50} for CGRP observed in our isolated coronary artery studies is well below the plasma levels of CGRP (80pM) observed in external jugular venous blood during a migraine attack (Goadsby and Edvinsson, 1993), making it unlikely that cranially-derived CGRP released by trigeminal activation during a migraine attack might affect coronary vascular tone.

The antagonist activity of telcagepant in coronary artery tissues was proven by our biochemical assays in which telcagepant reduced the increase in cAMP levels elicited by αCGRP, but not the cAMP increase elicited by forskolin, indicating that the effects of telcagepant were due to specific blockade of CGRP receptors rather than a non-specific mechanism affecting second messenger levels. This response was observed only in distal but not proximal segments, probably reflecting the relatively high number of proximal tissues that were unresponsive to CGRP. In our functional assays in coronary arteries, telcagepant

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antagonized the vasodilatation induced by αCGRP in a competitive manner as previously shown for its effects in cerebral and meningeal arteries (Edvinsson et al 2010). The potency of telcagepant was almost the same in distal coronary arteries and coronary arterioles (pA_2 of 8.43 ± 0.24, pK_b of 7.89 ± 0.13, respectively), despite the fact that the vasorelaxant effect of CGRP was larger in distal coronary vessels. The potency of telcagepant in coronary arteries seemed somewhat lower than in cerebral arteries (pA_2 of 9.37 ± 0.17), but was comparable to that in meningeal arteries (pK_b of 8.03 ± 0.16) (Edvinsson et al., 2010). When the potency of telcagepant is compared with that of other CGRP receptor antagonists in human coronary arteries, telcagepant results to be more potent than $CGRP_{8-37}$ and an early-generation small molecule CGRP receptor antagonist Compound 1 (Edvinsson et al., 2001; Hasbak et al., 2003). Telcagepant displays a slightly lower potency than the IV anti-migraine CGRP receptor antagonist olcegepant in coronary (pA_2 of 9.37) (Edvinsson et al., 2001; Gupta et al., 2006b), cerebral (Edvinsson et al., 2002) and middle meningeal (Gupta et al., 2006a) (dura mater) arteries.

Studies with the prototypic peptide CGRP receptor antagonist $CGRP_{8-37}$ (Paolocci et al., 2001) and the small molecule CGRP receptor antagonist olcegepant suggested that endogenous CGRP does not play a significant role in cardiovascular regulation under resting conditions, since these agents do not induce vasoconstriction in vitro (Gupta et al., 2006a; Gupta et al., 2006b) nor alter baseline hemodynamics in animals (Arulmani et al., 2004). It has however been hypothesized that CGRP is released as part of a protective mechanism under pathophysiological conditions such as ischemia (Li and Peng, 2002; Chai et al., 2006; Li et al., 2008). Since telcagepant is equipotent in cranial and coronary arteries this raised the question as to whether CGRP receptor antagonism by telcagepant during the treatment of migraine could impair such a response. A complicating factor in this assessment is that

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telcagepant is highly species-dependent with regard to binding affinity to the CGRP receptor, with significantly lower affinities in rat and dog than in non-human primate and human (Salvatore et al, 2008). This precludes assessment of its hemodynamic effects in common preclinical species. There is nonetheless a significant literature from studies with CGRP receptor antagonists lacking species specificity that can affect this debate. Thus, *in vitro* studies in isolated mouse and rat hearts have reported no effect of the prototype CGRP receptor peptide antagonist $CGRP_{8-37}$ and the small molecule CGRP receptor antagonist olcegepant on ischemic injury or function (Lu et al, 1999; Wang and Wang, 2005; Chai et al, 2006; Zhong and Wang, 2007). Some *in vitro* studies have reported that CGRP receptor antagonism attenuates ischemic preconditioning cardioprotection in isolated mouse and rat hearts, elicited by a set program of ischemia-reperfusion cycles preceding longer periods of myocardial ischemia (Lu et al, 1999; Chai et al, 2006; Zhong and Wang, 2007), but the pathophysiological significance of these observations is uncertain.

In contrast to studies *in vitro*, preclinical *in vivo* studies in multiple species with $CGRP_{8-37}$ and olcegepant have reported no intrinsic hemodynamic effects. Specifically regarding coronary function, *in vivo* hemodynamic studies in normal dogs have reported no effect of $CGRP_{8-37}$ on coronary or myocardial regional blood flow in dogs (Shen et al, 2001) and no effect of the small molecule CGRP receptor antagonist olcegepant on myocardial vascular conductance in normal rat and pig (Kapoor et al, 2003; Arulmani et al, 2004). Also, *in vivo* ischemia/reperfusion studies in rat and pig reported that $CGRP_{8-37}$ and olcegepant had no effect on infarct size (Kallner et al, 1998, Wu et al, 2001). Moreover, $CGRP_{8-37}$ had no effect on myocardial blood flow in dogs with heart failure produced by previous myocardial infarction and rapid ventricular pacing (Shen et al, 2003).

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In the current studies a strong contractile response was observed with the 5-$HT_{1B/1D}$ receptor agonist zolmitriptan in coronary artery segments consistent with earlier findings with all members of the serotonin agonist class of anti-migraine drugs in healthy (MaassenVanDenBrink et al., 1998) and diseased coronary arteries (Edvinsson and Uddman, 2005). A recent series of *in vivo* preclinical studies has compared the effects of the 5-$HT_{1B/1D}$ receptor agonist anti-migraine drug sumatriptan to the effects of CGRP or CGRP receptor antagonists on coronary vascular function in dogs in the settings of acute regional myocardial as well as coronary reactive hyperemia. During acute regional myocardial ischemia induced by atrial pacing in the presence of coronary stenosis, neither CGRP nor $CGRP_{8-37}$ affected coronary flow and severity of ischemia, whereas sumatriptan exacerbated ischemia severity with concomitant reduction in coronary blood flow (Regan et al, 2009; Lynch et al, 2009a). Likewise, $CGRP_{8-37}$ had no effect on myocardial reactive hyperemic response following brief mechanical coronary artery occlusion, whereas sumatriptan reduced peak reactive hyperemic coronary artery blood flow, reactive hyperemic flow and the repayment of coronary blood flow debt (Lynch et al 2009b). These preclinical findings indicate that caution should be exercised in the use of triptans in migraine patients with cardiovascular disease; further, they are in agreement with the outcome of recent studies showing that telcagepant did not exacerbate spontaneous ischemia in a small cohort of patients with stable coronary artery disease (Belm et al, 2008).

In conclusion, our findings *in vitro* suggest that telcagepant is unlikely to cause coronary side effects under normal physiological conditions in cardiovascular healthy patients. Telcagepant is currently being tested in migraine patients with stable vascular disease (Edvinsson and Linde, 2010; NCT00662818 at www.clinicaltrials.gov). The absence of vasoconstriction with

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telcagepant suggests a potential cardiovascular safety advantage of the CGRP antagonist class of anti-migraine agents as compared to the triptans.

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Footnotes

*) Both authors contributed equally

Legends for figures

Figure 1: Relaxant effect of αCGRP on human proximal (upper left panel) and distal (middle left panel) coronary arteries, as well as on human coronary arterioles (lower left panel). Concentration-response curves to αCGRP were constructed in the absence or presence of 300 nM (proximal), 30 nM - 1 μM (distal) and 1 μM (arterioles) of telcagepant (left panels). Right panels show the average Schild plot of the concentration response curves with the apparent pK_B value, calculated with a slope constrained to unity for the proximal coronary arteries (upper right panel) and coronary arterioles (lower right panel). The middle right panel shows the average Schild plot of the concentration response curves with the corresponding pA_2 in the distal coronary arteries. Values given represent mean ± S.E.M., n = 6-8.

Figure 2. Contractile response to zolmitriptan and telcagepant in proximal coronary arteries (upper left panel), distal coronary arteries (upper right panel) and coronary arterioles (lower left panel) segments.

Comparison of the maximum relaxant response to αCGRP with the maximum contractile response to zolmitriptan in proximal coronary artery, distal coronary artery and coronary arteriole segments (lower right panel). Values given represent mean ± S.E.M., n = 5-7.

Figure 3: Effect of telcagepant (1 μM) on the increase in cAMP levels induced by αCGRP (1 μM, upper panels) or by forskolin (10 μM, lower panels) in human proximal (left panels) and distal (right panels) coronary arteries. Values given represent mean ± S.E.M., n = 4-10.

$p < 0.05$, * control vs αCGRP or forskolin -/+ telcagepant and † αCGRP vs αCGRP + telcagepant.

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Figure 4: Immunohistochemistry of human distal coronary artery (upper, i.d. = 600-1000 μm) and coronary arteriole (lower, i.d.= about 300 μm) segments. Antibodies for RAMP1 and CLR showed positive staining in the walls of the artery segments. Co-staining with actin antibody revealed the localization of the immunoreactions in the smooth muscle cells (merged, arrows). No staining in endothelium or in the adventitial layers. Marker, 100 μm.

Figure 5: Immunohistochemistry of human distal coronary artery (upper, i.d. = 600-1000 μm) and coronary arteriole (lower, i.d.= about 300 μm) segments. Antibodies for RAMP1 and CLR showed positive staining in the cytoplasm of the smooth muscle cells in walls of the artery. The receptor component co-localized (merged, arrows). DAPI, staining nuclei, is used in the merged pictures (blue). Marker, 100 μm (upper panel) and 50 μm (lower panel).

Figure 1

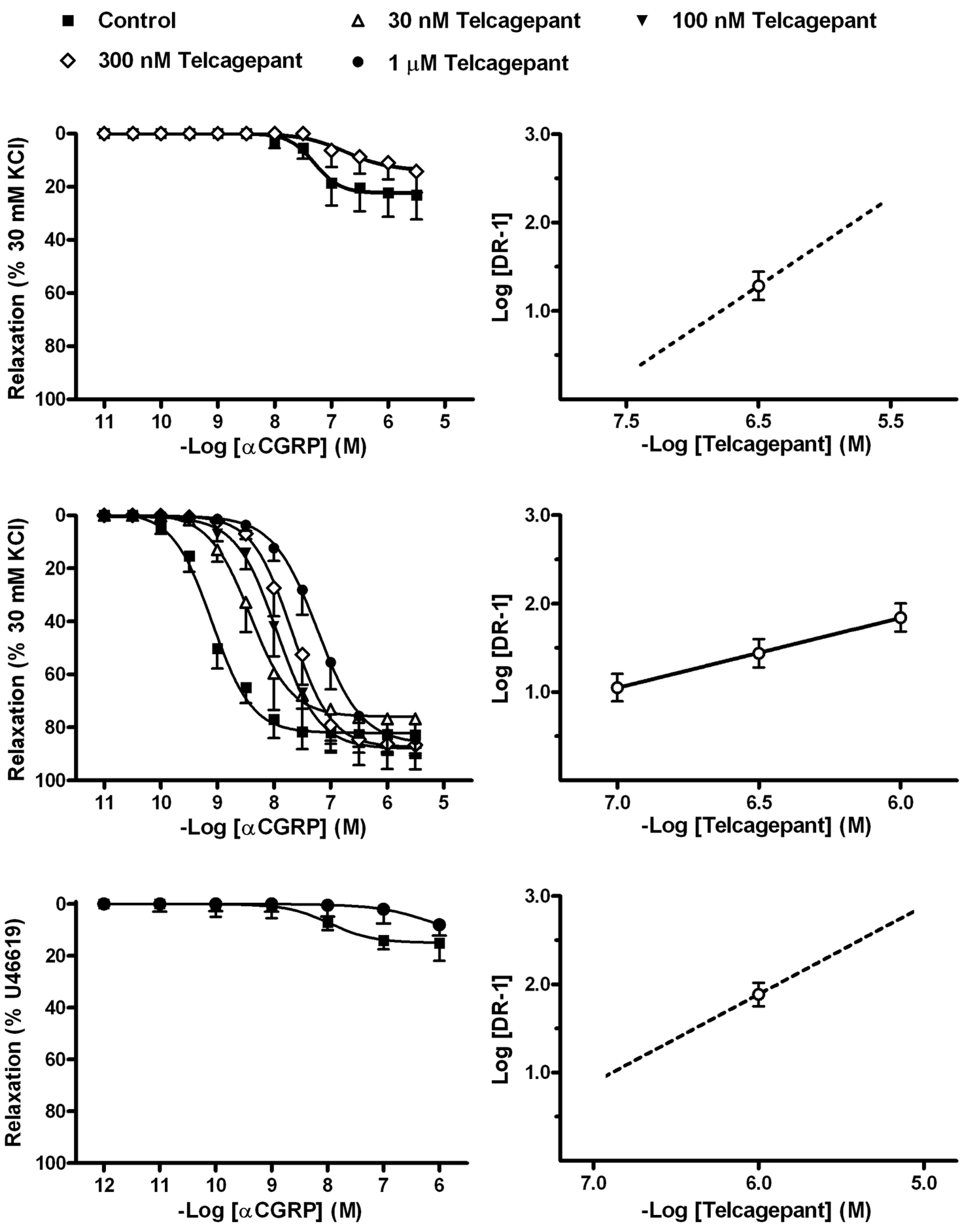


Figure 2

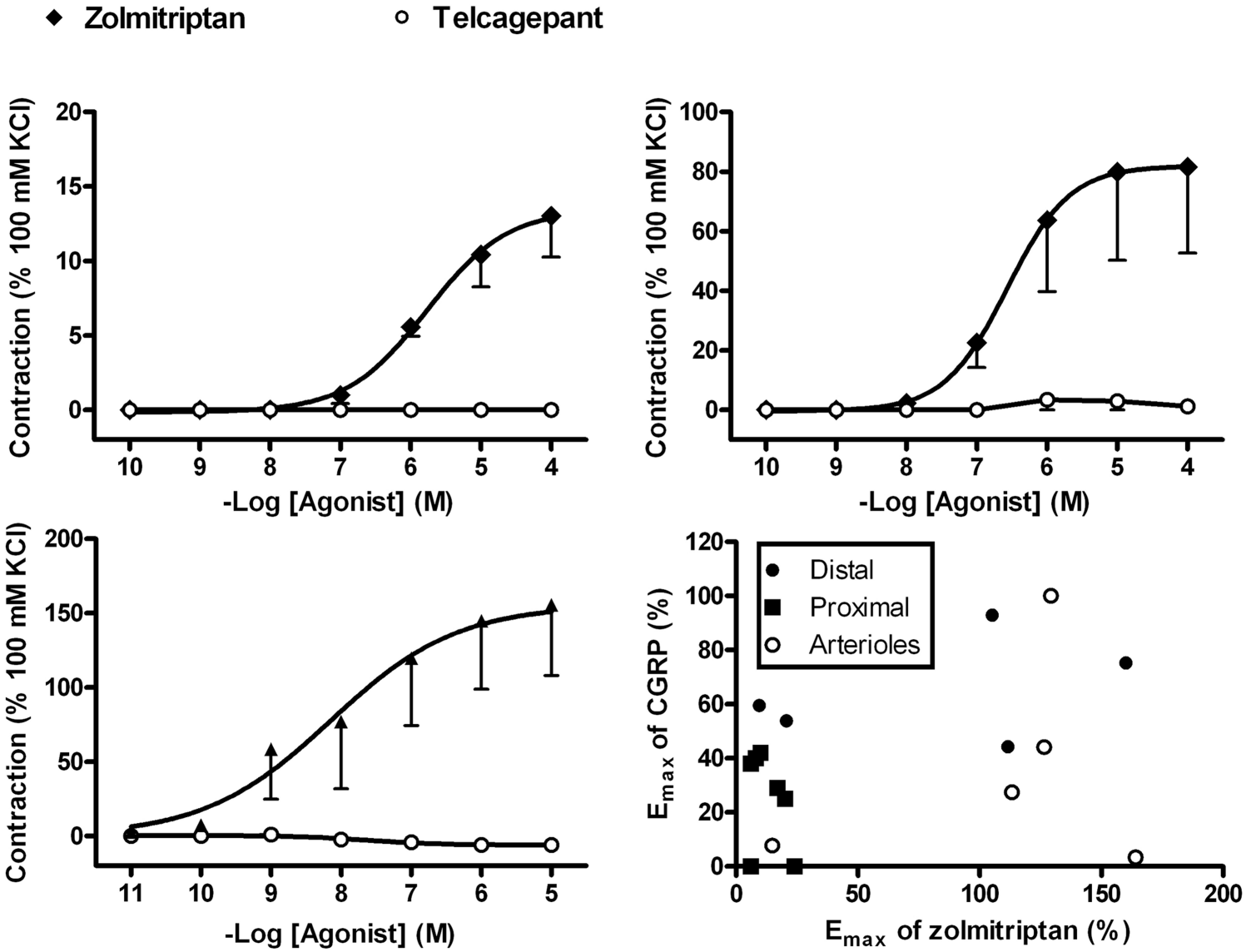
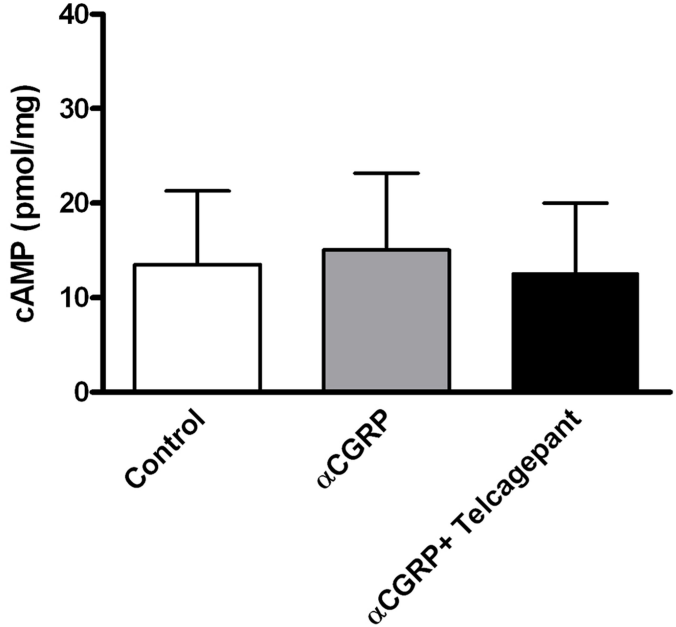
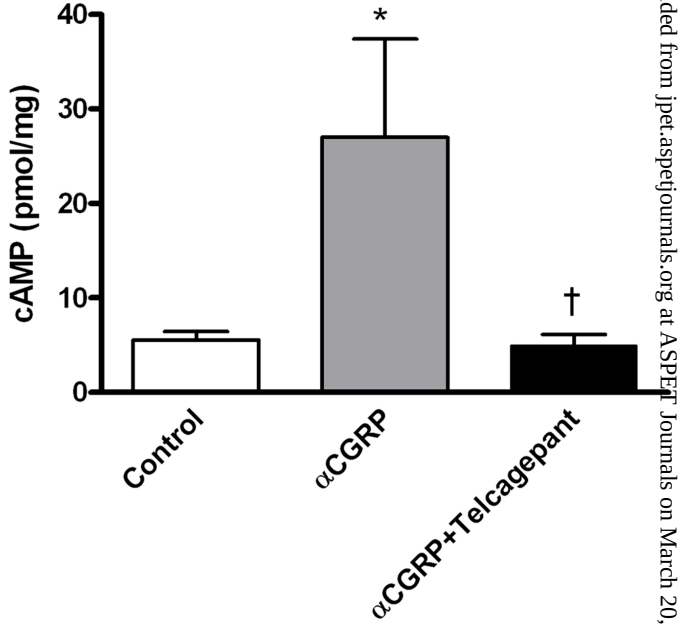
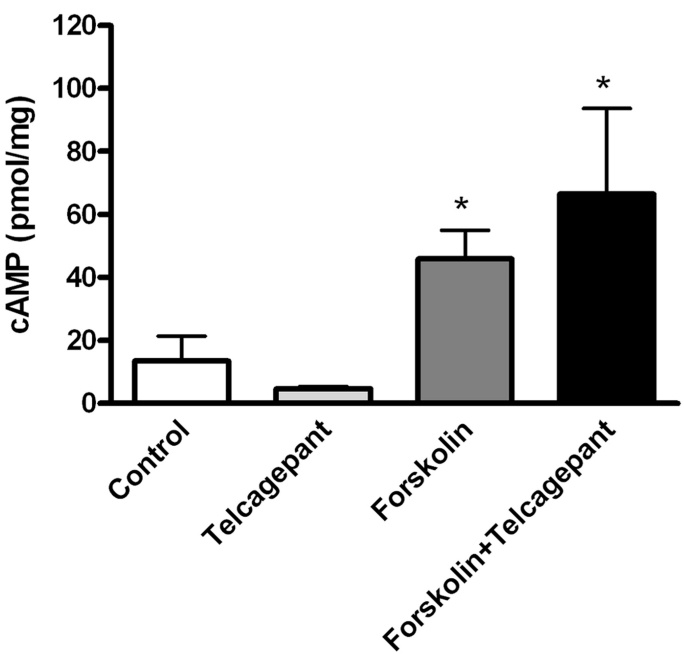


Figure 3







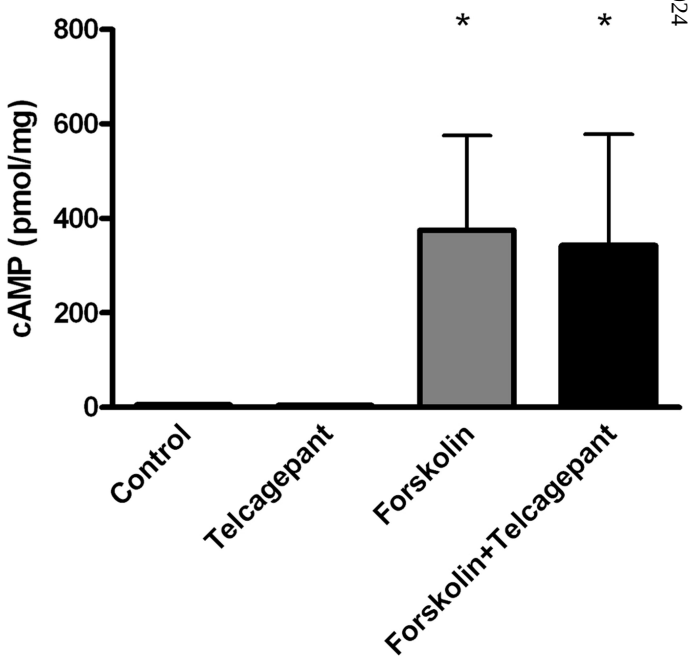


Figure 4

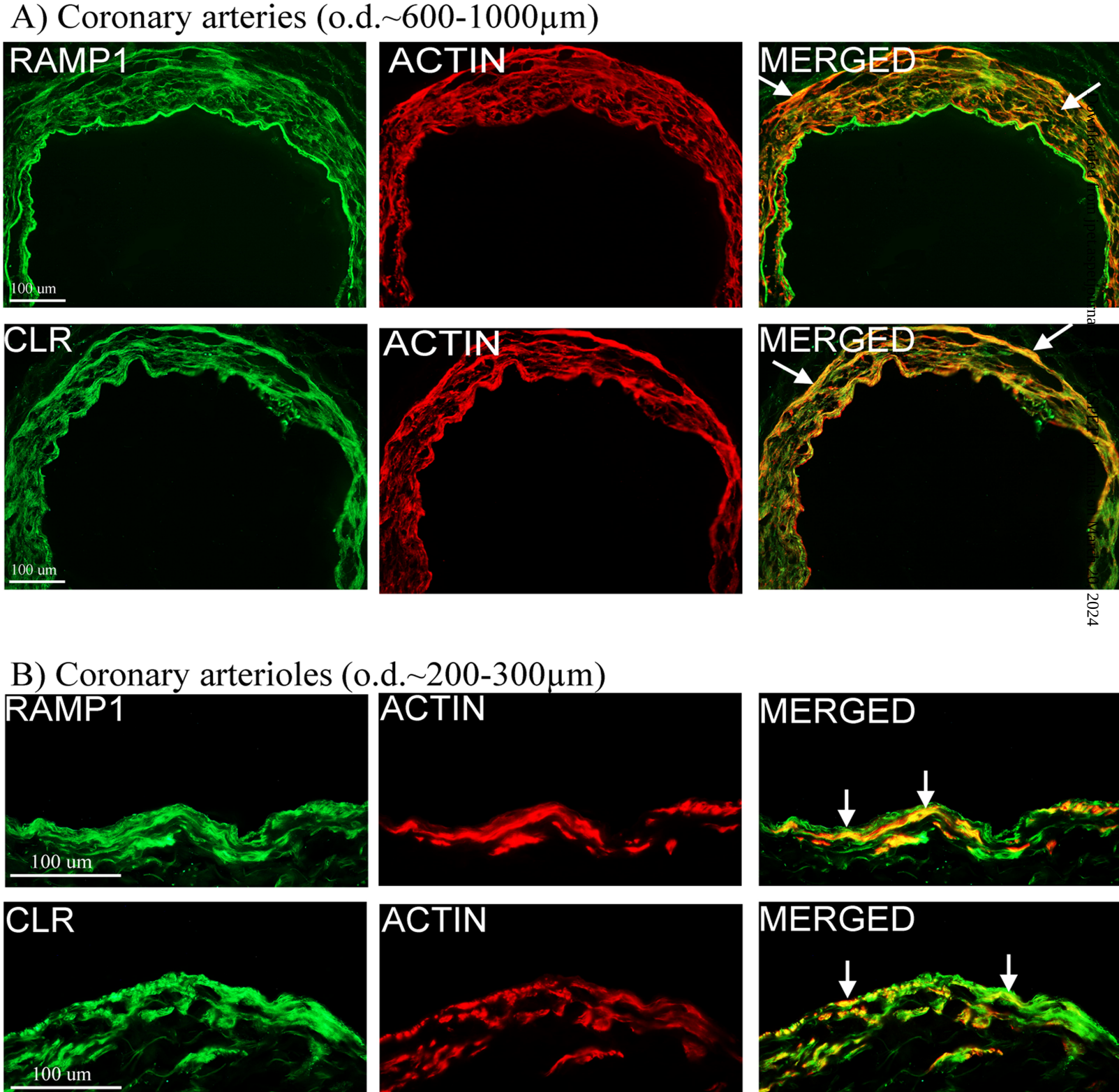

A) Coronary arteries (o.d.~600-1000µm)
RAMP1
ACTIN
MERGED
100 um
CLR
ACTIN
MERGED
100 um
B) Coronary arterioles (o.d.~200-300µm)
RAMP1
ACTIN
MERGED
100 um
CLR
ACTIN
MERGED
100 um

Figure 5