Characterization of the Calcitonin Gene-Related Peptide Receptor Antagonist Telcagepant (MK-0974) in Human Isolated Coronary Arteries


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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 antimigraine CGRP receptor antagonist telcagepant (MK-0974) [N-[(3R,6S)-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl]-4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridine-1-yl)piperidine-1-carboxamide; U46619, 9,11-dideoxy-11H-epoxy-9-carboxamido[4,5-b]imidazo[4,5-b]pyridine-1-yl)piperidine-1-carboxamide] on human isolated coronary arteries. Arteries with different internal diameters 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 coronary arteries (i.d. 2–3 mm; E\textsubscript{max} = 83 ± 7%) than proximal coronary arteries (i.d. 2–3 mm; E\textsubscript{max} = 23 ± 9%), coronary arteries from explanted hearts (i.d. 3–5 mm; E\textsubscript{max} = 11 ± 3%), and coronary arterioles (i.d. 200–300 μm; E\textsubscript{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 (p\textsubscript{A\textsubscript{2}} = 8.43 ± 0.24) and proximal coronary arteries and coronary arterioles (1 μM telcagepant, giving p\textsubscript{A\textsubscript{2}} = 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 colocalization of the CGRP receptor elements calcitonin-like receptor and receptor activity-modifying protein 1 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 antigranulocyte efficacy of the CGRP receptor antagonist olcegepant [BIBN4096BS; N-[2-[[5-amino-1-[[4-(4-pyridyl)-1-piperazinyl][carbonyl]penyl]amino]-1-[3-(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2H)-quinazolinyl)] 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) [N-[[3R,6S]-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepran-3-yl]-4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridine-1-yl)piperidine-1-carboxamide], a novel orally bioavailable antigranulocyte CGRP receptor antagonist (Paine 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 CGRP receptors in coronary vessels by using immunohistochemistry and compared the coronary vascular pharmacology of telcagepant with the vasoconstrictor antigranulocyte 5-HT1B/1D receptor agonist zolmitriptan in human coronary arteries of different diameters to assess its potential cardiovascular safety profile.

Materials and Methods

Human Isolated Arteries. The right proximal (i.d. 2–3 mm) and distal (i.d. 600–1000 μm) coronary arteries were obtained within 24 h after death from heart-beating organ donors (8 males, 11 females; ages 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; i.d. 3–5 mm) was removed in conjunction with coronary artery bypass surgery (five males, one female; ages 62–78 years); 2) epicardial arteries of somewhat larger diameter (i.d. 3–5 mm) were removed from two explanted hearts in conjunction with heart transplantation (one male, one female, ages 56 and 67 years, respectively), and 3) coronary arterioles of small diameter (approximately 300 μm) were removed during valvular surgery (five males, four females; ages 67–84 years). All vessels were placed in buffer solution aerated with 5% CO2 in O2 (carbogen) for transfer to the participating laboratories. The buffer composition in the Netherlands was 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, and 8.3 mM glucose, pH 7.4, and in Sweden the buffer composition was 119 mM NaCl, 4.7 mM KCl, 1.5 mM CaCl2, 1.17 mM MgSO4, 25 mM NaHCO3, 1.18 mM KH2PO4, 0.027 mM EDTA, and 5.5 mM glucose, pH 7.4. The Swedish study had the individual patients’ approval and was sanctioned by the Lund University Ethics Committee (LU99). 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 1 to 4 mm in 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 was connected 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 approximately 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 mm Hg (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 precontraction with prostaglandin F2α (1 μM, proximal coronary artery segments (Maassen-VanDenBrink et al., 1998)) or the thromboxane A2 analog U46619 (9,11-dideoxy-11α, 9α-epoxy-methano-prostaglandin F2α) (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 cCGRP 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 cCGRP, and the data were pooled for analysis because they were equipotent. Each segment was precontracted with 1 μM U46619 (Swedish experiments) or 30 mM KCl (Dutch experiments) before αCGRP was added. Pilot experiments (data not shown) showed that these different precontraction protocols did not affect the vasorelaxant (vasodilator) response to cCGRP. 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 to act as control (no antagonist present) and the other to test the agonist response after 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-hydroxytryptamine1B/1D (5-HT1B/1D) receptor agonist zolmitriptan were compared with contractile effects of telcagepant and the relaxations to cCGRP.

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 (0.5 mM) for 30 min in the absence or presence of telcagepant (1 μM; separate segments as those used for myograph experiments). The arterial segments were exposed to human cCGRP (10 μM) or forskolin (10 μM) for 5 min and then snap-frozen. Forskolin, which increases intracellular cAMP concentrations by activating adenyl cyclase, was used to assess the specificity of telcagepant toward CGRP-mediated increases in cAMP concentrations. The samples were stored at –80°C until cAMP assay by using an enzyme-linked immunosorbent assay kit and manual (R&D Systems Europe, Ltd., Abingdon, Oxfordshire, UK).

Compounds. The following materials were used in the in vitro experiments: human and rat cCGRP (NeoMPS S.A., Strasbourg, France, and Sigma-Aldrich, St. Louis, MO for the Dutch and Swedish experiments, respectively), prostaglandin F2α (tris salt), and U46619 (Sigma-Aldrich). Zolmitriptan (from nasal spray; AstraZeneca, London, UK or AK Scientific, Mountain View, CA, for the Swedish and Dutch experiments, respectively) was dissolved in saline. Tel-
cagepant (MK-0974) was synthesized and supplied by the Medicinal Chemistry Department (Merck, West Point, PA). αCGRP and U46619 were dissolved in water and stored as aliquots at −20°C. Telcagepant was dissolved in dimethyl sulfoxide 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 (= 100%). For each segment the maximum vasodilator effect ($E_{\text{max}}$) was calculated. The concentration-response curves for all compounds were analyzed by 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) by using Prism 4.0 (GraphPad Software Inc., San Diego, CA). The blocking potency of the antagonist was estimated by calculating EC$_{50}$ ratios and plotting a Schild plot (Arunlakshana and Schild, 1959) by using linear regression to get the slope value. In proximal coronary arteries and coronary arterioles, only one concentration of telcagepant was studied; in those cases “apparent pK$_A$” values were calculated, constraining the slope to unity. Because 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 those cases, the concentration-response curves were extrapolated, considering the maximal response in the absence of antagonist as $E_{\text{max}}$. Data are expressed as mean values ± S.E.M., 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, and correlations were used by using the SPSS 15.0 statistical program (SPSS Inc., Chicago, IL). The potency of telcagepant can be compared across the various arterial preparations because 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 (Invitrogen A/S, Taastrup, Denmark), frozen at −80°C, and subsequently sectioned into 10-μm-thick slices. Cryostat sections were fixed for 10 min 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 3 × 5 min. 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 rabbit anti-RAMP1 (1:50; Santa Cruz Biotechnology Inc., Santa Cruz, CA) or rabbit anti-CLR (1:100; Alpha Diagnostic International, San Antonio, TX). The primary antibodies were diluted in PBST, 1% bovine serum albumin, and 3% normal donkey serum. On the second day sections were brought to room temperature and rinsed in PBST for 3 × 15 min. Sections were subsequently incubated with secondary antibody (1 h, room temperature). The secondary antibody used was Cy2-conjugated donkey anti-rabbit (1:200; Jackson ImmunoResearch Laboratories Inc., West Grove, PA) diluted in PBST and 1% bovine serum albumin. The sections were subsequently washed with PBST and mounted with crystal mounting medium (Sigma-Aldrich). To determine cellular localization of RAMP1 and CLR, double immunofluorescence was performed by the addition of a mouse anti-smooth muscle actin antibody (1:200; Santa Cruz Biotechnology Inc.). As secondary antibody Texas red-conjugated donkey anti-mouse was used (1:200; Jackson ImmunoResearch Laboratories Inc.). To colocalize RAMP1 and CLR, an additional anti-goat CLR antibody was used (1:50; Santa Cruz Biotechnology Inc.). As secondary antibody Alexa Fluor 488 donkey anti-goat was used for the double staining (1:400; Invitrogen, La Jolla, CA). Vectashield medium (Vector Laboratories, Burlingame, CA) containing 4’, 6-diamidino-2-phenylindole staining nuclei was used on some sections.

Immunoreactivity was visualized with a BX 60 microscope (Olympus, Tokyo, Japan) at the appropriate wavelength. Photoshop CS3 (Adobe Systems, Mountain View, CA) was used to visualize colabeling 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 autofluorescence in lamina elastica interna was seen (not shown). As controls, to evaluate the autofluorescence in lamina elastica interna, preparations were made using only the primary antibodies.

Results

Functional Studies to αCGRP in Human Isolated Arteries. Precontracted proximal coronary arteries did not consistently relax to αCGRP in four of eight experiments. The mean $E_{\text{max}}$ to CGRP, including both responding and nonresponding arteries, amounted to 23 ± 9% of precontraction induced by 30 mM KCl ($n = 8$; Fig. 1, top left). The mean pEC$_{50}$ of the responding proximal coronary arteries was 7.2 ± 0.2. In coronary arteries obtained from two explanted hearts, which had somewhat larger diameter than the proximal coronary arteries from healthy heart-beating donors, αCGRP induced a small relaxation after precontraction with U46619 ($E_{\text{max}}$ of 11 ± 3% and pEC$_{50}$ of 8.1 ± 0.1). In distal coronary arteries αCGRP induced consistent relaxant responses with an $E_{\text{max}}$ of 83 ± 7% of precontraction induced by 30 mM KCl and a pEC$_{50}$ of 9.1 ± 0.1 ($n = 6$; Fig. 1, middle left). The small human coronary arteries obtained from patients undergoing valvular surgery relaxed upon administration of αCGRP in a consistent concentration-dependent manner with a smaller $E_{\text{max}}$ of 15 ± 7% and a pEC$_{50}$ of 8.0 ± 0.1 (Fig. 1, bottom left). 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 (Fig. 2). Pretreatment 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 coronary arteries (Fig. 1, top left) or distal coronary arteries (Fig. 1, middle left) and small coronary arterioles from valvular surgery patients (Fig. 1, bottom left). 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, $p = 0.07$) (Fig. 1, middle right). Although in the proximal coronary artery and the small coronary arterioles αCGRP was less potent and less efficacious as a relaxant agent compared with that seen in distal coronary arteries, telcagepant was still an effective antagonist in a consistent concentration-dependent manner with a smaller $E_{\text{max}}$ of 15 ± 7% and a pEC$_{50}$ of 8.0 ± 0.1 (Fig. 1, bottom left). There were no noticeable relaxant responses to αCGRP in segments of the LIMA (data not shown).

Effect of Zolmitriptan in Human Isolated Arteries. In proximal and distal coronary arteries zolmitriptan induced a concentration-dependent contraction, which was larger in distal segments ($99 ± 44%$; $n = 4$) than in proximal segments ($13 ± 3%$; $n = 7$) (Fig. 2, top). In the coronary arterioles zolmitriptan induced a strong concentration-dependent contraction ($155 ± 47%$; $n = 4$; Fig. 2, bottom left). All of these vessel segments also responded to αCGRP with relaxation; the $E_{\text{max}}$ of zolmitriptan was unrelated to that of αCGRP (Pearson’s correlation <0.4 and $p > 0.05$; Fig. 2, bottom right).
Effect of \(\alpha\)CGRP and Telcagepant on cAMP Levels.

In proximal coronary arteries \(\alpha\)CGRP, either in the absence or presence of telcagepant, did not increase cAMP levels (Fig. 3, top left). In contrast, \(\alpha\)CGRP increased cAMP levels in distal coronary arteries, which was abolished by pretreatment with telcagepant (Fig. 3, top right). Forskolin increased the cAMP levels in both proximal and distal coronary arteries; this increase was not inhibited by pretreatment with tel-
Telcagepant (Fig. 3, bottom). 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 (Fig. 4A) and coronary arterioles (Fig. 4B) 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 colocalization. With the use of another CLR antibody, we could verify that the two receptor components, RAMP1 and CLR, colocalized, supporting the presence of functional CGRP receptor in these arteries (Fig. 5). There were no obvious positive immunoreactions in the endothelium or the lamina elastica interna; the latter is strongly autofluorescent, especially in the green wavelength.

**Fig. 3.** Effect of telcagepant (1 μM) on the increase in cAMP levels induced by αCGRP (1 μM; top) or forskolin (10 μM; bottom) in human proximal (left) and distal (right) coronary arteries. Values given represent mean ± S.E.M. (n = 4–10). p < 0.05, *, control versus αCGRP or forskolin, †, αCGRP versus αCGRP + telcagepant.

**Fig. 4.** Immunohistochemistry of segments of human distal coronary artery (A; i.d. = 600–1000 μm) and coronary arteriole (B; i.d. = approximately 300 μm). Antibodies for RAMP1 and CLR showed positive staining in the walls of the artery segments. Costaining with actin antibody revealed the localization of the immunoreactions in the smooth muscle cells (merged, arrows). There is no staining in the endothelium or the adventitial layers. Scale bar, 100 μm.

**Fig. 5.** Immunohistochemistry of segments of human distal coronary artery (A; i.d. = 600–1000 μm) and coronary arteriole (B; i.d. = approximately 300 μm). Antibodies for RAMP1 and CLR showed positive staining in the cytoplasm of the smooth muscle cells in walls of the artery. The receptor component colocalized (merged, arrows). 4',6-Diamidino-2-phenylindole, staining nuclei, is used in the merged pictures (blue). Scale bars, 100 μm (A) and 50 μm (B).

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 colocalization. With the use of another CLR antibody, we could verify that the two receptor components, RAMP1 and CLR, colocalized, supporting the presence of functional CGRP receptor in these arteries (Fig. 5). There were no obvious positive immunoreactions in the endothelium or the lamina elastica interna; the latter is strongly autofluorescent, especially in the green wavelength.

**Discussion**

In the current studies, CGRP-induced concentration-dependent relaxations varied in magnitude in coronary arteries...
of different calibers 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 compared with proximal coronary arteries, coronary arteries from explanted hearts, and small coronary arteries. 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 caused by differences in CGRP receptor density between arteries but may reflect differences in efficiency of intracellular receptor coupling. It is noteworthy that the EC\textsubscript{50} for CGRP observed in our isolated coronary artery studies is well below the plasma levels of CGRP (80 pM) 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 cCGRP, but not the cAMP increase elicited by forskolin, indicating that the effects of telcagepant were caused by specific blockade of CGRP receptors rather than a nonspecific mechanism affecting second-messenger levels. This response was observed 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 antagonized the vasodilatation induced by cCGRP in a competitive manner as shown previously 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\textsubscript{2} of 8.43 ± 0.24 and pK\textsubscript{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\textsubscript{2} of 9.37 ± 0.17), but was comparable with that in meningeal arteries (pK\textsubscript{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 is more potent than CGRP\textsubscript{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 intravenous antimigraine CGRP receptor antagonist ocegepant in coronary arteries (pA\textsubscript{2} of 9.37) (Edvinsson et al., 2001; Gupta et al., 2006b), cerebral arteries (Edvinsson et al., 2002), and middle meningeal arteries (Gupta et al., 2006a) (dura mater).

Studies with the prototypic peptide CGRP receptor antagonist CGRP\textsubscript{8–37} (Paolocci et al., 2001) and the small-molecule CGRP receptor antagonist ocegepant suggested that endogenous CGRP does not play a significant role in cardiovascular regulation under resting conditions, because these agents do not induce vasoconstriction in vitro (Gupta et al., 2006a,b) or 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). Because 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 telcagepant is highly species-dependent with regard to binding affinity to the CGRP receptor, with significantly lower affinities in rat and dog than in nonhuman primate and human (Salvatore et al., 2008). This precludes assessment of its hemodynamic effects in common preclinical species. There is nonetheless 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\textsubscript{8–37} and the small-molecule CGRP receptor antagonist ocegepant 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\textsubscript{8–37} and ocegepant have reported no intrinsic hemodynamic effects. Specifically regarding coronary function, in vivo hemodynamic studies in normal dogs have reported no effect of CGRP\textsubscript{8–37} on coronary or myocardial regional blood flow (Shen et al., 2001), and no effect of the small-molecule CGRP receptor antagonist ocegepant on myocardial vascular conductance has been reported in normal rat and pig (Kapoor et al., 2003; Arulmani et al., 2004). In addition, in vivo ischemia/reperfusion studies in rat and pig reported that CGRP\textsubscript{8–37} and ocegepant had no effect on infarct size (Källner et al., 1998, Wu et al., 2001). Moreover, CGRP\textsubscript{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).

In the current studies a strong contractile response was observed with the 5-HT\textsubscript{1B/1D} receptor agonist zolmitriptan in coronary artery segments consistent with earlier findings with all members of the serotonin agonist class of antimigraine drugs in healthy coronary arteries (MaassenVanDenBrink et al., 1998) and diseased coronary arteries (Edvinsson et al., 2005). A recent series of in vivo preclinical studies has compared the effects of the 5-HT\textsubscript{1B/1D} receptor agonist antimigraine drug sumatriptan with the effects of CGRP or CGRP receptor antagonists on coronary vascular function in dogs in the settings of acute regional myocardial and coronary reactive hyperemia. During acute regional myocardial ischemia induced by atrial pacing in the presence of coronary stenosis, CGRP or CGRP\textsubscript{8–37} did not affect coronary flow and severity of ischemia, whereas sumatriptan exacerbated ischemia severity with concomitant reduction in coronary blood flow (Lynch et al., 2009b; Regan et al., 2009). Likewise, CGRP\textsubscript{8–37} had no effect on myocardial reactive hyperemic response after 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., 2009a). 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 (Behm et al., 2008).

In conclusion, our findings in vitro suggest that telcagepant is unlikely to cause coronary side effects under normal physiological conditions in healthy cardiovascular 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 vasodisconstriction with telcagepant suggests a potential cardiovascular safety advantage of the CGRP receptor antagonist class of antimigraine agents compared with the triptans.

**References**


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