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Effects of the prototype serotonin 5-$HT_{1B/1D}$ receptor agonist sumatriptan and the calcitonin gene-related peptide (CGRP) receptor antagonist $CGRP_{8-37}$ on myocardial reactive hyperemic response in conscious dogs

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ABSTRACT

The triptans, serotonin 5-$HT_{1B/1D}$ receptor agonists exemplified by sumatriptan, are a mainstay migraine therapy but have class labeling contraindicating their use in patients with coronary artery disease. Triptans constrict human coronary artery *in vitro*, and there are case reports of myocardial infarction in patients using sumatriptan. However, preclinical studies with sumatriptan in normal dogs have failed to demonstrate effects on resting coronary flow. Calcitonin gene-related peptide (CGRP) receptor antagonism, exemplified by the prototype CGRP receptor antagonist peptide $CGRP_{8-37}$, is a new antimigraine mechanism which also has been reported to have no effect on coronary flow in normal, non-stressed animals. The goal of the present studies was to compare the effects of sumatriptan (10 μg/kg/min i.v.) and $CGRP_{8-37}$ (30 μg/kg/min i.v.) on systemic and coronary hemodynamics in conscious dogs under resting conditions and during myocardial reactive hyperemia following a brief 15 s of coronary artery occlusion. Neither $CGRP_{8-37}$ nor sumatriptan affected resting coronary flow. However, whereas $CGRP_{8-37}$ had no effect on myocardial reactive hyperemic response, sumatriptan reduced peak reactive hyperemic coronary artery blood flow (baseline vs treatment: 75.4 ± 12.7 vs 60.0 ± 10.3 ml/min, $P<0.05$), reactive hyperemic flow (16.7 ± 5.2 vs 11.6 ± 3.3 ml, $P<0.05$) and the repayment of coronary blood flow debt following coronary artery occlusion (484 ± 76 vs $369 \pm 57\%$, $P<0.05$), indicating an impairment in coronary blood flow reserve. The positive control nitric oxide synthase inhibitor L-NNA (30 mg/kg/30 min i.v.) likewise significantly attenuated myocardial reactive hyperemic response. These findings provide evidence for a differentiation between CGRP receptor antagonism and triptan effects on coronary vascular function.

1. Introduction

Triptans, serotonin subtype 5-$HT_{1B/1D}$ receptor agonists exemplified by the prototype sumatriptan, are a mainstay treatment for migraine but have class labeling contraindicating their use in patients with confirmed or suspected coronary artery disease. Concern regarding the cardiovascular safety of sumatriptan, and the class in general, stems from the ability of triptans to constrict human coronary artery *in vitro* (Bax et al., 1993; Longmore et al., 1997; MaassenVanDenBrink et al., 1998), and case reports of myocardial ischemia and infarction in patients using sumatriptan (Ottervanger et al., 1993; O'Connor and Gladstone, 1995; Mueller et al., 1996; Erbilen et al., 2005; Anghileri et al., 2006). However, preclinical *in vivo* hemodynamic studies with sumatriptan primarily in normal non-stressed dogs have failed to demonstrate any effects on coronary circulation (Feniuk et al., 1989; Gupta et al., 1995; Parsons et al., 1997).

Calcitonin gene-related peptide (CGRP) receptor antagonism recently has emerged as a mechanistically novel antimigraine strategy (Goadsby, 2008; Durham, 2008; Tepper and Stillman, 2008). This mechanism has been validated through the demonstration of clinical efficacy with the parenteral CGRP receptor antagonist olcegepant (BIBN4096BS) (Olesen et al., 2004) and the orally available CGRP receptor antagonist MK-0974 (telcagepant) (Ho et al., 2008a,b). Preclinical *in vivo* hemodynamic studies in multiple species under non-stressed conditions with the prototype CGRP receptor peptide antagonist $CGRP_{8-37}$ (Chiba et al., 1989) and olcegepant have reported no effects on coronary or myocardial regional blood flow (Shen et al., 2001; Kapoor et al., 2003; Arulmani et al., 2004).

Recent studies have assessed the effects of sumatriptan and $CGRP_{8-37}$ in the setting of active regional myocardial ischemia produced by rapid atrial pacing in the presence of coronary artery stenosis in anesthetized dog (Regan et al., 2009; Lynch et al., 2009). These studies demonstrated a differentiation between these two agents, with sumatriptan exacerbating the severity of regional ischemia concomitant with a reduction in

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coronary blood flow (Lynch et al., 2009), whereas $CGRP_{8-37}$ had no effect on ischemia severity or coronary flow in the setting of cardiac stress (Regan et al., 2009). The present studies extended the comparison of the coronary effects of CGRP receptor antagonism and triptans by investigating the effects of $CGRP_{8-37}$ and sumatriptan on systemic and coronary vascular dynamics in chronically-instrumented conscious dogs. Specifically, the effects of these agents on resting coronary blood flow and myocardial reactive hyperemia in response to a brief 15 s occlusion of the coronary artery were determined. Additionally, the nitric oxide synthase inhibitor (N_ω-nitro-L-arginine) (L-NNA) was tested as a positive control (Puybasset et al., 1996; Andrieu et al., 1999).

The results of the present studies demonstrated that neither $CGRP_{8-37}$ nor sumatriptan affected resting, non-stressed coronary flow. However, whereas $CGRP_{8-37}$ had no effect on myocardial reactive hyperemic response, sumatriptan reduced peak reactive hyperemic coronary artery blood flow, reactive hyperemic flow and the repayment of coronary blood flow debt following coronary artery occlusion. As expected, L-NNA also reduced myocardial reactive hyperemic response. These findings constitute further differentiation between CGRP receptor antagonism and triptan effects on coronary function.

2. Materials and methods

All animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996, NIH Publ. No. 80-23) and were approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories, West Point, Pennsylvania.

2.1. Surgical preparation

Adult dogs (four beagles and two mongrel dogs of either sex, weighing 10–20 kg) were anesthetized with thiopental sodium (12–15 mg/kg i.v.). Following tracheal intubation and ventilation, general anesthesia was maintained with isoflurane (1.5–2.0 vol.% in oxygen). A left thoracotomy was performed at the fourth intercostal space. Tygon catheters (Norton Plastics, Akron, OH) were implanted in the descending aorta and left atrium for measurement of arterial and left atrial pressures, respectively. A solid-state miniature pressure gauge (Konigsberg, Pasadena, CA) was implanted in the left ventricular (LV) cavity through the apex for measurements of LV systolic pressure and rate of change of LV pressure (LV d*P*/d*t*). The left circumflex (LCX) coronary artery was isolated, and a hydraulic occluder made from Tygon tubing was placed around the vessel to induce a brief, complete occlusion for the measurement of myocardial reactive hyperemic response. Blood volume flow probes (Transonic Systems, Ithaca, NY) were also placed around the LCX coronary artery proximal to the occluder to continuously measure coronary blood flow. The chest was closed in layers and evacuated of air.

2.2. Measurement of hemodynamic parameters

Hemodynamic recordings were made using a data tape recorder (TEAC, Montebello, CA) and a multiple-channel oscillograph (Gould, Cleveland, OH). Arterial pressure was measured using strain gauge manometers (Argon, Athens, TX), which were previously calibrated using a mercury manometer connected to the fluid-filled catheters. The LV pressure gauge was cross-calibrated with aortic and left atrial pressure measurements. LV d*P*/d*t* was obtained by electronically differentiating the LV pressure signal. A triangular wave signal was substituted for the pressure signals to directly calibrate the differentiator (Triton Inc., San Diego, CA). Coronary blood flow was measured by using a volume flow meter (Transonic Systems Inc., Ithaca, NY). Mean arterial pressure and blood flow were measured by using an amplifier filter. A cardiotachometer, triggered by the ventricular pressure signal, provided instantaneous and continuous measurements of heart rate. Coronary vascular resistance was calculated as the quotient of mean arterial pressure and mean coronary blood flow. Peak reactive hyperemic coronary artery blood flow was determined following 15 s occlusions of the LCX coronary artery. Total coronary blood flow during reactive hyperemia also was measured by using a planimeter to integrate the area of the mean coronary blood flow recording. Coronary blood flow debt, reactive hyperemic flow, and coronary blood flow debt repayment were calculated as described previously (Shen et al., 2000). The equations used were as follows: coronary blood flow debt (ml) = control blood flow rate (ml/s) × duration of occlusion (s); reactive hyperemic flow (ml) = total blood flow during reactive hyperemia (ml) − [control blood flow rate (ml/s) × duration of reactive hyperemia (s)]; repayment of coronary blood flow debt (%) = [reactive hyperemic flow (ml) / coronary blood flow debt (ml)] × 100. The schematic depicted in Fig. 1 graphically represents and defines the myocardial reactive hyperemia parameters cited above.

2.3. Experimental protocol

Experiments were conducted 2–3 weeks after surgery with the dogs conscious and lying quietly on their right sides. Studies were conducted in cross-over manner, with all animals receiving all three treatments in random order following a minimum 2 days wash-out period. Baseline hemodynamic values as well as baseline coronary artery reactive hyperemic response were measured prior to intravenous test agent infusion. The baseline reactive hyperemic response, which was induced by occlusion of the LCX coronary artery for 15 s, was performed 3–5 times at 5 min intervals when the basal LV systolic pressure, LV d*P*/d*t*, mean arterial pressure, heart rate, and resting phasic and mean coronary blood flow were stable. The data then were averaged to obtain a baseline response to reactive hyperemia. Post-treatment hemodynamic values and coronary reactive hyperemic responses then were repeated with the following three treatments: a 10 min continuous i.v. infusion of 30 μg/kg/min $CGRP_{8-37}$, a 10 min continuous i.v. infusion of 10 μg/kg/min sumatriptan, or following a 30 min continuous i.v. infusion of total dose 30 mg/kg L-NNA (N_ω-nitro-L-arginine). Experiments with $CGRP_{8-37}$ and sumatriptan were conducted in 6 conscious dogs, while experiments with L-NNA were conducted in 5 dogs. Due to a technical failure in the latter group, LV pressure data from one L-NNA-treated animal were not obtained. Previous literature studies assessing myocardial reactive hyperemic responses in dogs typically have utilized durations of coronary occlusion ranging from 5 to 30 s. It should be noted that many previous studies have utilized multiple durations of occlusion within a study; conversely many studies have utilized single durations of coronary occlusion. The 15 s duration of coronary occlusion used in the

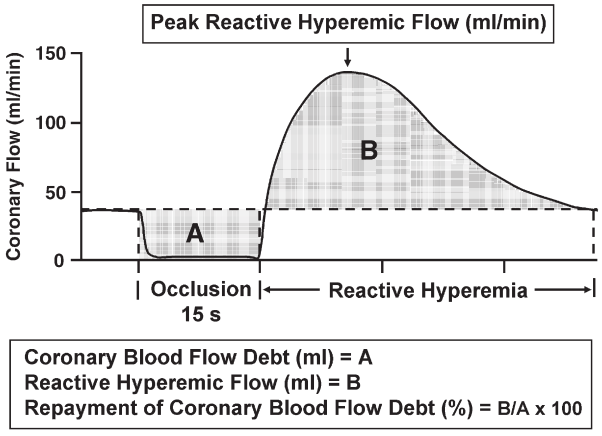

Fig. 1. Schematic representation of the left circumflex (LCX) coronary artery reactive hyperemic response to a 15 s occlusion, with measured and derived parameters indicated (see Section 2.2).