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Original article

A calcium channel blocker amlodipine increases coronary blood flow via both adenosine- and NO-dependent mechanisms in ischemic hearts

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Abstract

Amlodipine reduces oxidative stress that decreases NO and adenosine release. This study was undertaken to examine whether amlodipine mediates coronary vasodilation and improves myocardial metabolism and contractility in ischemic hearts via either adenosine- or NO-dependent mechanisms. In open-chest dogs, amlodipine (2 μg kg per min) was infused at the minimum dose that caused maximal coronary vasodilation. The perfusion pressure was reduced in the left anterior descending coronary artery so that coronary blood flow (CBF) decreased by 50%. Amlodipine increased the difference of the adenosine level (VAD (Ado): 119 ± 14 to 281 ± 46 nM) and the nitrate + nitrite level (VAD (NOx): 7.8 ± 1.3 to 16.1 ± 1.1 μM) between coronary venous and coronary arterial blood, and also increased CBF (50 ± 3 to 69 ± 6 ml/100 g/min). These changes were partially reversed by either 8-sulfophenyeltheophylline (8SPT) or L$^{\omega}$-nitro arginine methyl ester (L-NAME), and were completely blocked by both 8SPT and L-NAME. The reduction of CBF increased VAD (8-iso-prostaglandin $F_{2\alpha}$), and this increase was reduced by amlodipine (10.8 ± 1.1 to 5.0 ± 0.5 pg/ml). In addition, pretreatment with superoxide dismutase mimicked the coronary effects of amlodipine and blunted the response to amlodipine administration. Amlodipine-induced coronary vasodilation via both adenosine- and NO-dependent mechanisms. Adenosine and NO may interact in ischemic hearts to mediate coronary vasodilation by amlodipine.

Keywords: Calcium channel blocker; Adenosine; Ischemia; Coronary circulation

1. Introduction

Ca channel blockers are often used for the treatment of ischemic heart disease, because coronary vasodilation [1,2] is promoted via inhibition of Ca^{2+} entry into smooth muscle cells [3]. Long-acting dihydropyridine Ca channel blockers were recently reported to protect the endothelium of renal resistance arteries in hypertensive rats [4] and the mesenteric arteries in rats with circulatory shock [5]. Interestingly, amlodipine increases NO production by coronary arterial endothelial cells [6], and we have reported that other long-acting Ca channel blockers (benidipine and nifedipine) have the potential to increase NO production in ischemic heart [7,8]. Since oxidative stress inactivates NO and amlodipine suppresses oxidative stress [9,10], this drug may promote NO release. On the other hand, we have reported that oxidative stress inactivates ecto-5′-nucleotidase [11,12], the enzyme

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responsible for adenosine production, because it is located on the cell membrane and may be targeted by oxygen-derived free radicals. These lines of evidence support the hypothesis that amlodipine increases coronary blood flow (CBF) in ischemic myocardium via both adenosine- or NO-dependent mechanisms.

The aim of this study was to determine whether 1) amlodipine increases either adenosine or NO release from ischemic myocardium in canine hearts, and 2) whether coronary vasodilation mediated by amlodipine is attenuated by either 8-sulfophenyltheophylline (8SPT) or L$^{\omega}$-nitro arginine methyl ester (L-NAME). To approach the goal, we examined the difference in the concentrations of adenosine (VAD (Ado)) and nitrate + nitrite (VAD (NOx)) between coronary venous blood and coronary arterial blood during infusion of amlodipine with 8SPT or L-NAME treatment. Furthermore, we tested whether amlodipine could increase VAD (Ado), VAD (NOx), and CBF in the presence of superoxide dismutase (SOD). We showed that amlodipine could increase both adenosine and NO release in ischemic canine myocardium, thus contributing to coronary vasodilation. We also detected a role of amlodipine-induced attenuation of oxygen-derived free radical generation in these actions.

2. Materials and methods

2.1. Instrumentation [13]

The hybrid dogs (HBD) mated with the Beagle, the American Fox Hound and the Labrador Retriever for the laboratory use (body weight: 15–21 kg) were anesthetized by an intravenous injection of sodium pentobarbital (30 mg/kg), intubated, and ventilated using room air mixed with oxygen (100% O_2 at a flow rate of 1.0–1.5 l/min). The chest was opened through the left fifth intercostal space, and the heart was suspended a pericardial cradle. After intravenous administration of heparin (500 U/kg), the left anterior descending (LAD) coronary artery was cannulated and perfused with blood from the left carotid artery through an extracorporeal bypass tube. CBF in the perfused region was measured with an electromagnetic flow probe attached to the bypass tube, and coronary perfusion pressure (CPP) was monitored at the tip of the coronary artery cannula. We obtained coronary vascular resistance (CVR) by calculation of CPP/CBP.

A thin (1 mm) and short (70 mm) cannula connected to a thin tube was inserted into a small coronary vein near the center of the perfused region to sample coronary venous blood. The draining venous blood was collected in a reservoir at the level of the left atrium and then was returned to the jugular vein. Hydration was maintained by slow infusion of normal saline. The pH, pO_2, and pCO_2 of systemic arterial blood before the protocol was 7.39 ± 0.02, 106 ± 3, and 38.0 ± 2.0 mmHg, respectively. A pair of ultrasonic crystals was placed at the inner one-third of the myocardium about 10 mm apart in order to measure myocardial segment length with an ultrasonic dimension gauge (5 MHz, 2 mm in diameter; Schuessler, Cardiff-by-the Sea, CA). Hemodynamic parameters were recorded on a multi-channel recorder (Rm-6000; Nihon Kohden, Tokyo, Japan). End-diastolic length (EDL) was measured at the R wave of the electrocardiogram and end-systolic length (ESL) was measured at the minimal value of the first derivative of left ventricular pressure. Then fractional shortening (FS) was calculated as [(EDL – ESL)/EDL] × 100%.

Agents were administered into the LAD via the bypass tube. We purchased sodium pentobarbital, heparin, 8SPT, L-NAME, adenosine, bradykinin, and SOD from Sigma (St. Louis, MO, USA). Amlodipine was kindly provided by Pfizer Pharmaceutical Inc. (Sandwich, UK). The investigation conforms with the *Guide for the Care and Use Laboratory Animals* published by the US National Institutes of Health (NIH Publication NO. 85-23, revised 1996).

2.2. Experimental protocols

2.2.1. Protocol I

After hemodynamic stabilization, coronary arterial and venous blood were sampled for blood gas analysis and for measurement of the lactate, nitrate + nitrite [14], and adenosine [15] concentrations, allowing the calculation of VAD (NOx), VAD (Ado), myocardial oxygen consumption. Myocardial oxygen consumption (ml/100 g/min) is calculated by CBF (ml/100 g/min) × the oxygen difference between coronary arterial and venous blood (ml/dl).

Lactate concentrations were measured by an enzymatic assay, and lactate extraction ratio (LER) was calculated as the coronary arteriovenous difference of the lactate concentration multiplied by 100 and divided by the arterial lactate concentration.

We used 29 dogs for Protocol I. Hemodynamic parameters (systolic and diastolic aortic blood pressure, and heart rate) were monitored. To examine whether either adenosine- or NO-dependent mechanisms were involved in amlodipine-induced coronary vasodilation in ischemic heart, we infused saline (n = 9), 8SPT (25 μg/kg per min at an infusion rate of 0.0167 ml/kg per min and a concentration of 1.5 mg/ml, n = 5), L-NAME (10 μg/kg per min at an infusion rate of 0.0167 ml/kg per min and a concentration of 0.6 mg/ml, n = 5), or L-NAME + 8SPT (n = 5) into the bypass. We dissolved amlodipine in 0.15% DMSO with saline and either L-NAME or 8SPT in saline. After we confirmed that either systemic or coronary hemodynamics did not change after 5 min of the infusion, we reduced CPP so that CBF decreased to 50% of the baseline value for 5 min. Then amlodipine (2 μg/kg per min at an infusion rate of 0.0167 ml/kg per min and a concentration of 0.12 mg/ml) was infused and continued for 120 min while CPP was maintained at the set low constant value. As for the control of the amlodipine treatment, we infused the solvent of amlodipine (at an infusion rate of 0.0167 ml/kg per min and a concentration of 0.12 mg/ml, n = 5). The times for the measurements of coro-