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Vasoconstrictor prostanoids may be involved in reduced coronary reactive hyperemia after ischemia–reperfusion in anesthetized goats

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Abstract

To examine coronary vasodilator reserve after ischemia–reperfusion, reactive hyperemia was determined during reperfusion after partial and total, brief and prolonged ischemia. To this, left circumflex coronary artery flow was electromagnetically measured, and partial (60 min) or total (15 and 60 min) occlusions of this artery were induced, followed in each case by 60-min reperfusion in anesthetized goats untreated and treated with N^{W}-nitro-L-arginine methyl ester (L-NAME) or meclofenamate. In untreated and treated animals, coronary flow was decreased during reperfusion after the three types of ischemia. In hyperemic responses to 5- and 10-s coronary occlusions, repayment of debt decreased during reperfusion after the three types of ischemia in untreated animals, and this decrease was not affected by L-NAME. This decrease during reperfusion after partial and total, 60-min ischemia, but not after total, 15-min ischemia, reversed with meclofenamate. Peak hyperemic flow/control flow ratio decreased only during reperfusion after total 60-min occlusion in untreated animals and it was normalized by meclofenamate. These results show that ischemia–reperfusion reduces hyperemic response (vasodilator reserve); this diminution being dependent on duration and severity of ischemia. The hyperemic responses reduction during reperfusion after prolonged ischemia, but not after brief ischemia may be related at least in part to increased production of vasoconstrictor prostanoids.

Keywords: Coronary blood flow; Coronary vasodilator reserve; Myocardial ischemia; Vasoconstrictor prostanoid; Nitric oxide

1. Introduction

Ischemia–reperfusion is a clinical and experimental event that can produce dysfunction of coronary vessels in addition to dysfunction of the myocardium. Coronary vascular function after ischemia–reperfusion has been studied by examining coronary vasodilator capacity or reserve, and the experimental data provide contradictory results. It may be related, in part, that the index used to evaluate vasodilator reserve as endothelium-dependent vasodilatation may be depressed, whereas endothelium-independent vasodilatation in response to vasodilator drugs is not altered during reperfusion after partial ischemia (Headrick et al., 1990; Nichols et al., 1994), and after total, brief ischemia (Kim et al., 1992; Richard et al., 1994) or total, prolonged ischemia (Kim et al., 1992; Hagar, 1994). Studies using reactive hyperemia as an index of coronary vasodilator reserve show that peak hyperemic flow is depressed during reperfusion after 1 h of subtotal ischemia (Nichols et al., 1994), and that it may be depressed (Nicklas and Gips, 1989) or not (Jeremy et al., 1989) during reperfusion after total, 10- or 15-min ischemia, and that it is depressed during reperfusion after total, 1-h ischemia (Nichols et al., 1988). In one of these studies, it is reported that repayment of debt is also decreased during reperfusion after total, 15-min ischemia (Nicklas and Gips, 1989). On the other hand, mechanisms underlying the decreased coronary vasodilator reserve after ischemia–reperfusion remain uncertain, and endothelial dysfunction with decreased nitric oxide release (Nichols et al., 1988) and altered production of prostanoids (Rubanyi and Vanhoutte, 1985) have been involved. The involvement of prostanoids as a pathogenic basis of reduced vasodilator reserve is attractive, as the production of vasoconstrictor prostanoids such as thromboxane A_2 may be increased after ischemia–reperfusion (Coker et al.,

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1981). Thromboxane A_2 is a potent vasoconstrictor (Coleman et al., 1990), and the coronary effects of a thromboxane analog are augmented after ischemia–reperfusion (Nichols et al., 1994). Prostacyclin and thromboxane A_2 can be produced by the myocardium during ischemia–reperfusion, and the evolution of the myocardium affected by ischemia–reperfusion may be determined by the balance between the production of thromboxane A_2 and prostacyclin during this situation, as thromboxane A_2 can produce detrimental effects whereas prostacyclin can produce beneficial effects on the myocardium affected. Studies using selective inhibitors of thromboxane A_2 synthase or thromboxane A_2 receptor antagonists suggest that vasoconstrictor prostanoids are involved in myocardial and vascular dysfunction after ischemia–reperfusion (Schrör and Thiemermann, 1986; Mullane and Fornabaio, 1988; Nichols et al., 1989). However, it has been reported that cyclooxygenase inhibitors have little protection against myocardial damage (Ogletree and Lefer, 1976; Bolli et al., 1981) and loss of vasodilator reserve as tested by determining the peak reactive hyperemia (Nichols et al., 1988) or the vasodilator response to acetylcholine and bradykinin (Nichols et al., 1988; Mehta et al., 1989). This absence of beneficial effects of cyclooxygenase inhibitors could be related to the indices used to evaluate the vasodilator reserve, and to the duration and/or severity of ischemia that precedes to reperfusion. There are data showing that myocardial accumulation of arachidonic acid during ischemia is a relatively slow process and the duration of the preceding ischemic period strongly influences the amount of arachidonic acid accumulated during the reperfusion phase (Van Bilsen et al., 1989), and that thromboxane A_2 may not be produced during reperfusion if the ischemic period is <60 min (Engels et al., 1990). Therefore, we have examined the effects of meclofenamate, an inhibitor of cyclooxygenase, on the action of reperfusion after different degrees of ischemia on reactive hyperemia, which was evaluated by determining peak hyperemic flow/control flow ratio and repayment of debt. This permits the analyzation of two parameters of reactive hyperemia that may be mediated by different factors (Kumaru et al., 2000). Although it has been tested that thromboxane A_2 is involved in the decreased vasodilator reserve after ischemia–reperfusion, the use of cyclooxygenase inhibitors permits to analyze the possible participation of vasodilator prostanoids (prostacyclin) and vasoconstrictor prostanoids (thromboxane A_2 and prostaglandins).

In our laboratory we have recently reported that coronary vasodilator reserve as tested with acetylcholine and sodium nitroprusside is preserved during reperfusion after partial ischemia (Fernández et al., 2002), and after total, brief ischemia but not after total, prolonged ischemia (Fernández et al., 2003). In some animals of these previous experiments, we found that hyperemic responses were decreased after ischemia–reperfusion and that this decrement reversed with meclofenamate. These preliminary observations prompted us to complete them with new experiments. Thus, the present study was performed to examine the effects of reperfusion after partial and after total, brief (15 min) and prolonged (60 min) ischemia on coronary reactive hyperemia, analyzing the role of prostanoids, as well as that of nitric oxide in these effects. The experiments were carried out in anesthetized goats where the left circumflex coronary artery flow was electromagnetically measured, and hyperemic responses after 5- and 10-s coronary occlusions were recorded under control conditions and during reperfusion after the three types of coronary ischemia. This was performed in animals untreated, treated with the inhibitor of cyclooxygenase, meclofenamate, and treated with the inhibitor of nitric oxide synthesis, N^{W}-nitro-L-arginine methyl ester (L-NAME).

2. Methods

2.1. Experimental preparation

In this study, 48 adult female goats (30–57 kg) were used. Anesthesia of the animals was induced with intramuscular injection of 10 mg/kg ketamine hydrochloride and i.v. administration of 2% thiopental sodium; supplemental doses were given as necessary for maintenance. After orotracheal intubation, artificial respiration with room air was instituted by use of a Harvard respirator. A left thoracotomy in the fourth intercostal space was performed and the pericardium was opened. The proximal segment of the left circumflex coronary artery was dissected, and an electromagnetic flow probe (Biotronex) was placed on this artery to measure blood flow. A snare-type occluder was also placed around the artery, distal to the flow probe, to obtain zero-flow baselines. Systemic arterial pressure was measured through a polyethylene catheter placed in one temporal artery and connected to a Statham transducer. In every animal, coronary flow, systemic arterial pressure and heart rate were simultaneously recorded on a Grass model 7 polygraph. Coronary vascular resistance was calculated by dividing mean systemic arterial pressure in mm Hg by coronary blood flow in milliliters per minute. Blood samples from the temporal artery were taken periodically to measure pH, pCO_2 and pO_2 by standard electrometric methods (Radiometer, ABL^{TM5}, Copenhagen, Denmark). After termination of the experiments, the goats were killed with an overdose of i.v. thiopental sodium and potassium chloride.

2.2. Experimental protocol

After the experimental preparation was ended and the hemodynamic variables had reached steady state, the hyperemic responses to 5- and 10-s coronary occlusions were tested three times each under control conditions (before ischemia) and after 60 min of reperfusion following ischemia in three groups of animals. Group a: Partial ischemia was performed in animals untreated (6 goats), treated with L-NAME (5 goats) and treated with meclofenamate (5 goats). In these experiments, hyperemic responses to 5- and 10-s coronary occlusions were tested under control conditions. Then, a critical, partial occlusion of the left circumflex coronary artery was achieved with another occluder which was variable and was placed around the artery immediately after the flow probe, so that this occluder was situated between the flow probe and the occluder used for obtaining