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Effect of ischemia duration and nitric oxide on coronary vasoconstriction after ischemia–reperfusion

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Abstract

The effects of the duration of ischemia on coronary vasoconstriction after ischemia–reperfusion were analysed in rat hearts. After 15, 30 or 45 min of global zero-flow ischemia and 15 min reperfusion, the coronary response to endothelin-1 (10^{-10}–10^{-7} M) and the thromboxane A2 analogue 9,11-dideoxy-1*a*,9*a*-epoxymethanoprostaglandin F2α (U46691, 10^{-8}–10^{-6} M) was recorded. Vasoconstriction induced by endothelin-1 only increased after short 15 min periods of ischemia. In contrast, the vasoconstriction induced by U46619 remained unmodified by short ischemias but was reduced after longer periods of ischemia (30 and 45 min). Inhibition of nitric oxide synthesis with the N^{w}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) augmented the vasoconstriction induced by endothelin-1 in non-ischemic hearts, but not following ischemia. Similarly, L-NAME increased the vasoconstriction induced by U46619 to a greater extent in non-ischemic hearts than following ischemia. These results suggest that ischemia–reperfusion inhibits nitric oxide production, causing an increased coronary response to endothelin-1 after brief ischemias. Longer ischemias may non-specifically inhibit coronary vasoconstriction and reduce nitric oxide production.

Keywords: Ischemia; Reperfusion; Endothelin; U46619; Vasoconstriction; NO (nitric oxide)

1. Introduction

Ischemia–reperfusion is a clinical and experimental event that can produce dysfunction of coronary vessels and dysfunction of the myocardium. The factors that determine the extent of this dysfunction include the duration and severity of the ischemia. The endothelium may release factors that produce vasodilation (nitric oxide, prostacyclin) or vasoconstriction (endothelin, thromboxane A2), and which may regulate vascular reactivity. There is evidence that these endothelial mechanisms are altered by ischemia–reperfusion, and that these endothelial alterations may contribute to the physiopathology of coronary ischemia. The release of endothelial nitric oxide and endothelium-dependent relaxation may be reduced after ischemia–reperfusion (Ku, 1982). Moreover, a number of studies suggest that the marked vasoconstriction produced by endothelin may be modulated by nitric oxide (Berti et al., 1993; Pernow and Modin, 1993). Endothelin may be involved in the deleterious effects of ischemia–reperfusion (Hagar, 1994) since the plasma levels of endothelin-1 are increased in acute myocardial ischemia (Miyauchi et al., 1989). Furthermore, the use of endothelin antagonists may have a beneficial effect in experimental myocardial ischemia (Grover et al., 1992).

The responsiveness of coronary circulation to vasoconstrictor stimuli may be altered after ischemia–reperfusion. However, these alterations may depend on the particular vasoconstrictor agent involved, and possibly on the experimental technique and species analysed. Coronary vasoconstriction mediated by endothelin-1 may be increased after ischemia–reperfusion in rats (Neubauer et

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al., 1991; Watts et al., 1992), pigs (Wang et al., 1995), dogs (Saito et al., 1992) and goats (Fernández et al., 2003), but it might not be modified after partial ischemia and reperfusion in coronary circulation of the goat (Fernández et al., 2002). Coronary vasoconstriction in response to 9,11-dideoxy-1*a*,9*a*-epoxymethanoprostaglandin F2α (U46619), 5-hydroxytryptamine or potassium is reduced after a 15 min ischemia in the perfused heart of rats (Hasan and McDonough, 1995). In contrast, the response to noradrenaline in the same preparation was no modified after 15 min ischemia (Pannangpetch and Woodman, 1996), although contraction following exposure to noradrenaline is augmented after chronic occlusion of the left circumflex coronary artery of the dog (Rapps et al., 1997). Contraction induced by 5-hydroxytryptamine increases after ischemia–reperfusion in porcine coronary arteries (Headrick et al., 1990) but it is reduced in the coronary circulation of dogs (Woodman, 1990). These inconsistencies may in part also be due to differences in the duration of ischemia, as long but not short ischemias increased the vasoconstriction to endothelin-1 in the coronary circulation of the goat (Fernández et al., 2003) or the pig (Lockowandt et al., 2001). Moreover, long but not short ischemias reduced the contraction induced by potassium in porcine coronary arteries (Dignan et al., 1995). As a result of this potential effect, we have analysed the influence of the duration of ischemia on the coronary vasoconstriction produced by endothelin-1 in the heart from rats perfused according to the Langendorff procedure, an experimental model frequently used for the study of ischemia–reperfusion (Sutherland and Hearse, 2000). We have also compared the response to endothelin-1 with that of another vasoconstrictor, such as the analogue of thromboxane A2 U46619. Finally, we have examined how nitric oxide modulates this vasoconstriction.

2. Methods

This study was carried out on 87 male Sprague–Dawley rats (weight 300–350 g) in compliance with applicable laws and regulations, as well as the principles expressed in the National Institute of Health's USPHS Guide for the Care and Use of Laboratory Animals. The use of these animals was approved by the University's Animal Care and Use Committee. The hearts were removed from the rats after their anaesthesia with pentobarbital sodium (40 mg/kg) and an injection of heparin (1000 IU). After obtaining the hearts, a cannula was inserted into the ascending aorta and retrograde perfusion of the heart was initiated with Krebs–Henseleit buffer (in mM: NaCl, 115; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11). This perfusion was performed in a non-recirculating Langendorff heart perfusion apparatus, at a constant flow of 11–15 ml/min to achieve a basal perfusion pressure of approximately 70 mmHg. The hearts were then paced at a rate of 240 beats/min by an electrical stimulator. Perfusion coronary pressure and left ventricular pressure (recorded with a latex balloon inflated to a diastolic pressure of 5–10 mmHg) were measured with Statham transducers and recorded in a Grass model 7 polygraph. The first derivation of the pressure (dP/dt) was calculated from the intraventricular pressure.

After a 15 min equilibration period with constant flow perfusion, the hearts were exposed to 15, 30 or 45 min of zero-flow ischemia, followed in each case by 15 min reperfusion at the same flow rate as before ischemia. In time-control hearts the flow rate of perfusion was maintained constant throughout. The coronary vasoconstriction in response to endothelin-1 (10^{-10}–10^{-7} M) or to U46619 (10^{-8}–10^{-6} M) was recorded in each heart after ischemia–reperfusion or in control time-matched perfusion. Endothelin-1 or U46619 were injected for 1 min at a constant rate using an infusion pump just proximal to the aortic cannula. In the case of U46619, each concentration was injected once the perfusion pressure had returned to the value registered at the time of the previous injection. As the vasoconstriction following endothelin-1 injection was persistent, each dose was injected when the response to the previous dose had reached a plateau.

To analyse the role of nitric oxide on the vasoconstictor responses in ischemia–reperfusion, the vasoconstriction induced by endothelin-1 or U46619 was recorded in the hearts after ischemia–reperfusion, in the presence or absence of the nitric oxide synthesis blocker N^{w}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M). The L-NAME was added to the perfusion solution 10 min before adding endothelin-1 or U46619. Therefore, the experiments were performed over the following time course: after 15 of perfusion, ischemia was produced during 15, 30 or 45 min. Perfusion was again initiated to produce reperfusion, and 5 min later L-NAME was added where necessary. The hearts were then perfused for 10 min longer in the presence or absence of L-NAME before registering the dose–response curve to endothelin-1 or U46619. In treated, non-ischemic hearts, L-NAME was added at a similar point in the experiment (10 min before registering the dose–response curves to endothelin-1 or U46619). Hemodynamic parameters were measured in all cases at an equivalent moment during the experiment, just before the beginning of the dose–response curves. The hemodynamic parameters and responses to endothelin-1 or U46619 were compared in ischemic hearts and non-ischemic hearts, exposed to L-NAME or not.

As the flow was maintained constant, the increases in perfusion pressure were considered as vasoconstriction and calculated as the mean±standard error of the mean (S.E.M.). The coronary responses in the different exper-

imental conditions were compared by analysis of variance (ANOVA), followed by Dunnet's test to determine the statistically significance of the comparisons.

The substances used were: endothelin-1 (human, porcine) from Bachem, 9,11-dideoxy-1*a*,9*a*-epoxymethanoprostaglandin F2α (U46619), and N^{w}-nitro-L-arginine methyl ester hydrochloride (L-NAME), both obtained from Sigma.

3. Results

After inducing ischemia for 15, 30 or 45 min, followed in each case by a 15 min reperfusion, systolic left ventricle pressure and systolic d*P*/d*t* were reduced when compared with non-ischemic hearts. The extent of this reduction was dependent on the duration of the ischemia. Furthermore, coronary perfusion pressure was significantly higher than in control hearts after the longest period of ischemia studied (45 min; Table 1). In non-ischemic hearts, coronary perfusion pressure was higher when they were pretreated with L-NAME (92±6 vs. 69±2.5 mmHg; $P<0.01$). However, no differences were observed in coronary perfusion pressure in L-NAME treated or untreated ischemic hearts (78±4.1 vs. 72±3.5; 83±2.1 vs. 80±3.6 and 115±6 vs. 125±7, for 15, 30 and 45 min periods of ischemia, respectively).

Injection of endothelin-1 (10^{-10}–10^{-7} M) produced concentration-dependent coronary vasoconstriction (Fig. 1). This vasoconstriction increased after the shortest ischemia (15 min) but was not modified by longer periods of ischemia (30 and 45 min), although a mild tendency towards a reduction was observed after 45 min ischemia. Inhibition of nitric oxide synthesis with L-NAME (10^{-4} M) increased the vasoconstriction induced by endothelin-1 in the control non-ischemic hearts, but not in ischemic–reperfused hearts for all periods of ischemia studied.

Likewise, U46619 (10^{-8}–10^{-6} M) produced dose-dependent coronary constriction (Fig. 2). This vasoconstriction was not modified after the shortest period of ischemia (15 min) but it was reduced when ischemia was of greater duration (30 and 45 min). In the control, non-ischemic hearts, treatment with L-NAME increased the vasoconstriction to the three doses of U46619 used, whereas following ischemia this treatment only increased the vasoconstriction at the highest dose of U46619 (10^{-6} M).

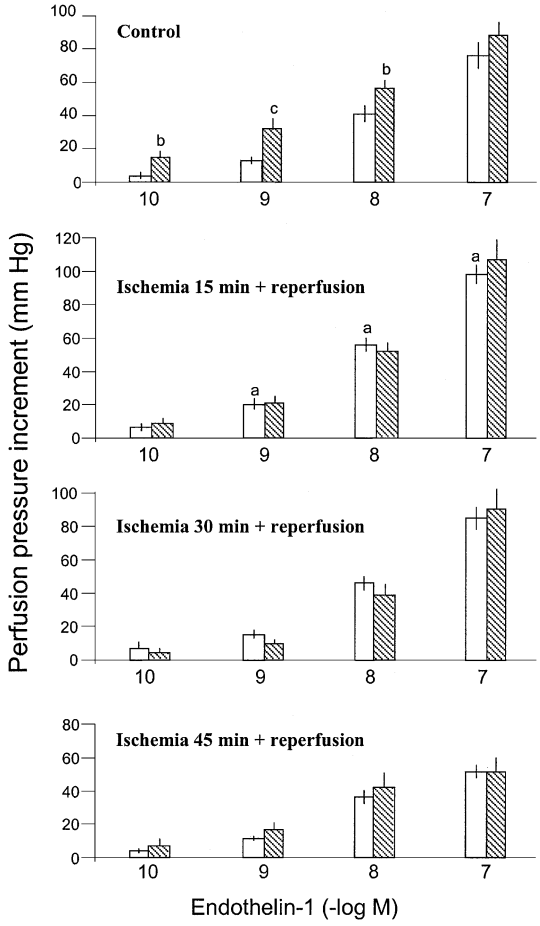

Fig. 1. Coronary vasoconstriction to endothelin-1 (10^{-10}–10^{-7} M) in rat perfused hearts under control conditions (n=6), and after 15 (n=6), 30 (n=6) and 45 (n=5) min ischemia and 15 of reperfusion, both in the presence (hatched bars) and absence (open bars) of L-NAME (10^{-4} M; n=6, n=5, n=5 and n=4, for control, and 15, 30 and 45 min ischemia, respectively). Data are represented as the mean±S.E.M. Significant difference between ischemic and non-ischemic hearts ([a]$P<0.05$) and between hearts treated and untreated with L-NAME ([b]$P<0.05$; [c]$P<0.01$).

Table 1
Hemodynamic parameters of perfused rat hearts in control conditions and after 15, 30 and 45 min ischemia, followed by 15 min reperfusion

	CPP (mmHg)	SVP (mmHg)	Systolic d*P*/d*t* (mmHg/s)
Control	69±2.5 (n=12)	89±5	2243±188
15 min ischemia	72±3.5 (n=13)	60±5[a]	1300±98a
30 min ischemia	80±3.6 (n=11)	41±4[a]	307±65[a]
45 min ischemia	125±7[a] (n=10)	35±3[a]	165±32[a]

CPP=coronary perfusion pressure; SVP=systolic intraventricular pressure. Values are means±S.E.M.

[a] Statistically significant compared to control ($P<0.01$).

4. Discussion

In this study, we found that ischemia followed by reperfusion in the perfused rat heart impaired contractility of the myocardium. Indeed, a reduction in intraventricular systolic pressure and d*P*/d*t* was observed that was directly related to the duration of the ischemia. Furthermore, the increase in the coronary perfusion pressure observed after the longest period of ischemia studied (45 min), indicated that coronary vascular resistance increased after ischemia–reperfusion. This corresponds to the phenomenon known as "non-reflow" (Kloner et al.,

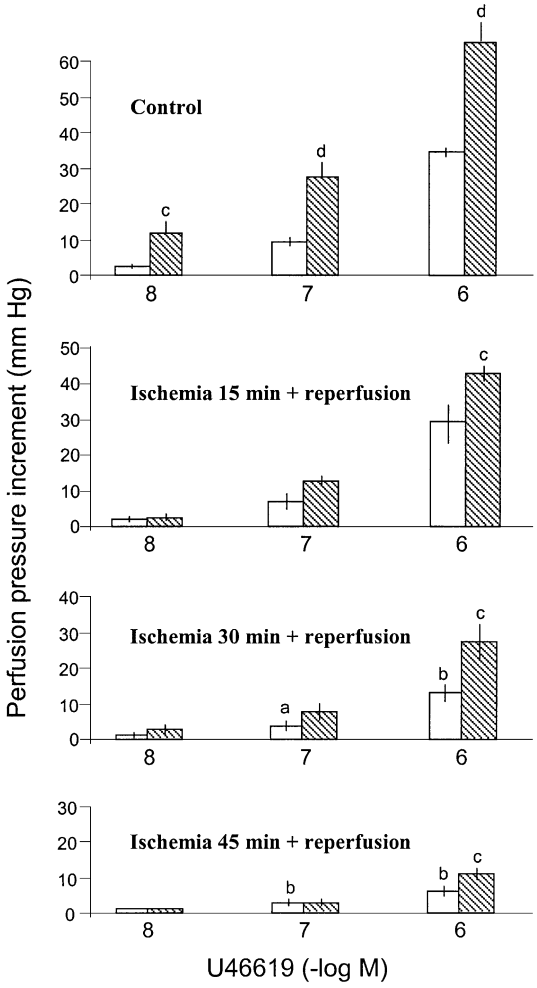

Fig. 2. Coronary vasoconstriction to U46619 (10^{-8}–10^{-6} M) in rat perfused hearts under control conditions (n=6), and after 15 (n=7), 30 (n=5) and 45 (n=5) min ischemia and 15 of reperfusion, in the presence (hatched bars) and absence (open bars) of L-NAME (10^{-4} M; n=5, n=5, n=6 and n=5, for control, and 15, 30 and 45 min ischemia, respectively). Data are represented as the mean±S.E.M. Significant difference between ischemic and non-ischemic hearts (^{a}P<0.05; ^{b}P<0.01), and between hearts treated and untreated with L-NAME (^{c}P<0.05; ^{d}P<0.01).

1974), which has been demonstrated both experimentally and clinically after coronary ischemia (Reffelmann and Kloner, 2002). Our results suggest that this phenomenon depends on the duration of the ischemia, since it was only observed after the longest periods of ischemia studied (45 min).

The inhibition of nitric oxide synthesis with L-NAME produced vasoconstriction, suggesting that a basal level of coronary vasodilation exists in this experimental model due to the release of nitric oxide. This is in accordance with other in vivo studies in which a basal vasodilator nitrergic tone in coronary circulation appears to exist (García et al., 1992; Bassenge, 1995). However, following ischemia L-NAME did not modify the resting vascular tone, indicative of a reduction in the basal release of nitric oxide. This inhibition of the nitrergic vasodilator tone may contribute to the increase in vascular resistance after ischemia–reperfusion. Indeed, an impairment of nitric oxide release after ischemia–reperfusion has been reported in several studies (see Laude et al., 2001). However, it seems unlikely that the reduced release of nitric oxide is the only cause of the non-reflow phenomenon in this experimental paradigm, particularly since the non-reflow phenomenon was not restored when the effect of L-NAME was abolished. Indeed, non-reflow was only evident after the longest period of ischemia (45 min) while no effect of L-NAME was seen after any period of ischemia studied. Thus, the mechanisms that underlie the non-reflow phenomenon remain unknown, although it appears that several factors are involved (Forman et al., 1989; Ku, 1982).

In agreement with earlier data (see Pernow and Wang, 1997), we have found that ischemia increased coronary vasoconstriction in response to endothelin-1 following ischemia–reperfusion, in this case even after relatively short periods of ischemia (15 min). In our experiments, this increase may at least in part be due to the reduction in nitric oxide production. In control conditions, coronary vasoconstriction induced by endothelin-1 is modulated by nitric oxide, as shown previously (García et al., 1996), and as confirmed by the effects of L-NAME on endothelin-1 induced constriction in control non-ischemic hearts. However, after 15 min of ischemia L-NAME no longer modifies the vasoconstriction induced by to endothelin-1, suggesting that this modulation by nitric oxide is abolished. Indeed, this may contribute to the increased response to endothelin-1 under these conditions. Longer periods of ischemia (30 and 45 min) did not modify the coronary vasoconstriction produced by endothelin-1, although in contrast to the shorter periods of ischemia (15 min) a tendency for weaker contractions was observed, albeit not fulfilling the criteria of statistical significance. However, nitric oxide production may also have been impaired after these longer periods of ischemia, as the contractions induced by endothelin-1 after 30 and 45 min of ischemia were not modified by L-NAME, as occurred after 15 min of ischemia. The absence of an increased coronary response to endothelin-1 after these longer periods of ischemia may be due to the impaired response of the coronary artery smooth muscle to this peptide. This effect would compensate for the potentiating effects of the reduction of nitric oxide production. The reduction of nitric oxide during ischemia–reperfusion has been described previously and may be related to inhibition by protein kinase C (Numaguchi et al., 1996) or to inactivation by oxidant radicals (Gross et al., 1992; Mehta et al., 1989).

The effects of U46619 in part coincide with those observed with endothelin-1. The coronary response to U46619 may also be modulated by nitric oxide and this influence of nitric oxide may also be impaired following ischemia–reperfusion. Indeed, L-NAME increased the vasoconstriction to every dose of U46619 used in control,

non-ischemic hearts. However, after short or long periods of ischemia, it increased the response only to the highest dose of U46619 (10^{-6} M). As with endothelin-1, long periods of ischemia may impair the vasoconstriction induced by U46619. This was reflected by the fact that in the presence of U46619, contractions were weaker after 30 and 45 min of ischemia than in control, non-ischemic hearts. Short 15 min periods of ischemia reduced the contractions in response to U46619 in the presence of L-NAME but not in its absence. This result suggests that after short periods of ischemia, the reduction in nitric oxide compensates for the impairment in vasoconstriction, as observed for endothelin-1. Therefore, we hypothesise that opposing effects are exerted on the coronary vasoconstrictor response by ischemia–reperfusion and that these influences may have different time courses.

Ischemia–reperfusion may rapidly reduce nitric oxide production (apparent after as little as 15 min), while longer periods of ischemia impair vasoconstriction through more marked mechanisms. With respect to U46619, the impairment of vasoconstriction and the reduction in nitric oxide compensate for each other over short periods of time (15 min), while when ischemia lasts longer the impairment of vasoconstriction predominates. For endothelin-1, nitric oxide reduction produces an increase in the response after 15 min of ischemia, yet after longer ischemic periods, the reduction in nitric oxide and the impairment of vasoconstriction compensate for each other. We do not know the cause of this reduction in vasoconstrictor capacity but it may be non-specific, as observed with both endothelin-1 and U46619, and it may be due to the impairment of contractile mechanisms. Furthermore, down-regulation of endothelin receptors due to the increased release of endothelin-1 during ischemia–reperfusion might be involved in the reduced response. Indeed, an increase in the release of endothelin-1 during ischemia–reperfusion in the perfused rat heart has been described previously (Brunner et al., 1992).

In summary, our results suggest that short and long term ischemia–reperfusion reduces nitric oxide production in coronary circulation, and that this causes an increase in the contraction produced by endothelin-1 after short periods of ischemia. Longer periods of ischemia may induce a non-specific decrease in the coronary vasoconstrictor capacity, in addition to reducing nitric oxide release. Understanding these processes is fundamental to be able to reduce the damage to coronary and myocardial tissue following ischemia.

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