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Interaction of adenosine and prostacyclin in coronary flow regulation after myocardial ischemia

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Abstract

The role of adenosine and prostacyclin in post-ischemic vasodilation was investigated using a model of sequential perfusion of two isolated hearts. Two guinea pig hearts were sequentially perfused (10 ml/min) without (control, $n = 4$) or with preceding 10-min ischemia ($n = 6$) of Heart I. Under control conditions no hemodynamic changes were observed in Heart II during sequential perfusion. After 10 min of ischemia of Heart I coronary perfusion pressure decreased by 23% in Heart II at the onset of sequential perfusion. Adenosine A_1 and A_2 receptor antagonists 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (2 μM) and 3,7-dimethyl-1-propargylxanthine (DMPX) (20 μM) infused simultaneously inhibited this decrease in coronary perfusion pressure by 74%, whereas indomethacin (5 μM) had no effect. DPCPX, DMPX and indomethacin in combination induced a significant increase in coronary perfusion pressure. Adenosine release (HPLC) into the coronary effluent after ischemia was significantly enhanced in the presence of indomethacin. These results suggest that after myocardial ischemia prostacyclin has an inhibitory effect on adenosine release.

Keywords: Ischemia; (Guinea pig); Vasodilation; Adenosine; Prostacyclin; DPCPX (1,3-dipropyl-8-cyclopentylxanthine); DMPX (3,7-dimethyl-1-propargylxanthine); Indomethacin

1. Introduction

Temporary occlusion of coronary vascular arteries is followed by coronary vasodilation, which is dependent in intensity on the duration of ischemia. This increase in coronary flow is a self-regulating mechanism that adjusts coronary perfusion of the heart to enhanced metabolic requirements after myocardial ischemia, and therefore plays a role in functional recovery of the myocardium (Bardenheuer and Schrader, 1986). The mechanisms of regulation of coronary vasomotion after myocardial ischemia and hypoxia represent complex phenomena which are still a matter of debate. With regard to the possible role of vasodilatory metabolites, reactive hyperemia in the heart after short-term occlusion appears to be mediated essentially by nitric oxide (NO) (Kostic and Schrader, 1992; Park et al., 1992; Chlopicki and Gryglewski, 1993; Hansen and Haunso, 1995) — whereas vasodilation after longer interruption of coronary flow has been attributed mainly to adenosine (Gryglewski et al., 1995b). Prostacyclin is also released into coronary circulation after myocardial ischemia during reperfusion, but its functional role in post-ischemic vasodilation is not yet clear (Engels et al., 1990; Gryglewski et al., 1995a).

In general, studies on the functional role of mediators released after myocardial ischemia are hampered by changes due to ischemia itself. Thus, chemical factors such as partial pressure of oxygen, carbon dioxide, tissue pH, as well as neurogenic and myogenic mechanisms, are also involved in modulating post-ischemic vasomotion (Blass et al., 1980).

The purpose of the present study was to investigate the role of adenosine and prostacyclin in post-ischemic vasodilation, independently of chemical and metabolic factors. Toward this objective we used a model of sequential

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perfusion consisting of two isolated guinea pig hearts (Felix et al., 1997; Stangl et al., 1997). This model allows to investigate the effects of mediators released after myocardial ischemia from an isolated heart, on a sequentially perfused non-ischemic second heart used as bioassay. Mediator-induced vasodilation was ascertained in the second recipient heart. In addition, the roles as well as interaction of prostacyclin and adenosine were further examined by using a cyclooxygenase inhibitor and selective adenosine receptor antagonists.

2. Methods

2.1. Isolated heart preparation: double heart model

The sequential perfusion of two isolated hearts was performed according to the technique as described by Schrader et al. for the guinea pig heart, and as further modified by us, as described previously (Schrader et al., 1977; Felix et al., 1997; Stangl et al., 1997). At the onset of serial perfusion, the coronary effluent of Heart I was reoxygenated by carbogen gas (95% O_2/5% CO_2) in a microchamber (500 μl) and rapidly transported to Heart II by a roller pump. Reduction of transit time to less than 3 s was achieved by miniaturization of the oxygenator and the connecting tubes (inner diameter (i.d.) = 0.5 mm). It was possible to realize different perfusion modes by means of a system of valves and pumps: separate perfusion at constant pressure (60 cm H_2O), separate perfusion at constant flow (10 ml/min), and sequential perfusion.

2.2. Experimental protocol (Fig. 1)

The hearts were separately perfused without intervention until stabilization at constant pressure (60 cm H_2O). Separate perfusion at constant flow (10 ml/min) was then initiated. This was followed by sequential perfusion without (Control, $n = 4$) or with preceding 10 min of ischemia (Ischemia, $n = 6$) of Heart I (Fig. 1). In addition, the following pharmacological interventions were performed to identify the role of adenosine and prostacyclin in post-ischemic vasoreactivity, and to elucidate the interaction of these two mediators.

2.2.1. Protocol 1

The first series of experiments was designed to investigate the role of adenosine in mediating post-ischemic vasodilation observed in the sequentially perfused, non-ischemic second heart. The selective adenosine A_1 receptor antagonist (1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 2 μM, $n = 6$) and the selective adenosine A_2 receptor antagonist (3,7-dimethyl-1-propargylxanthine (DMPX), 20 μM, $n = 6$) were intracoronarily infused into Heart II. The infusions were started 20 min prior to sequential perfusion in Heart II and were continued until the end of the experiment. Preliminary dose-finding studies established the amount of receptor antagonists. Administration of 2 μM DPCPX and 20 μM DMPX completely antagonized the effect of endogenous adenosine up to 1 μM.

In addition, the coronary effluent of the first heart was collected prior to and after 10 min of global ischemia, at measurement times of 0, 0.5, 1, 1.5 and 2 min, in order to determine adenosine release.

2.2.2. Protocol 2

The second series of experiments was performed to evaluate the role of prostacyclin release in post-ischemic vasodilation, as determined in the sequentially perfused, non-ischemic second heart. Indomethacin (5 μM, $n = 7$) was infused into the coronary circulation of Heart I and Heart II to inhibit endogenous prostacyclin formation. In Heart I, intracoronary infusion was started 15 min prior to global ischemia, stopped during ischemia, restarted at the onset of reperfusion and continued until the end of the experiment. In Heart II infusion of indomethacin was started 15 min before sequential perfusion and was stopped at the onset of sequential perfusion.

In addition, the coronary effluent of the first heart was collected prior to and after 10 min of global ischemia, at measurement times of 0, 0.5, 1, 1.5, 2, 3 and 5 min, in order to determine 6-keto-prostaglandin $F_{1\alpha}$ overflow.

2.2.3. Protocol 3

In a third series of experiments, both the selective adenosine A_1 and A_2 receptor antagonists and indomethacin were administered ($n = 7$). In the first heart, indomethacin infusion was started 15 min prior to ischemia, stopped during ischemia, restarted again at the onset of reperfusion, and continued until the end of the experiment. In the second heart, intracoronary infusion of indomethacin was started 15 min prior to sequential perfusion and was stopped at the onset of sequential perfusion. DPCPX (2 μM) and DMPX (20 μM) were intracoronarily infused into Heart II. Infusion of the receptor antagonists was initiated 20 min prior to sequential perfusion and continued until the end of the experiment.

2.2.4. Interaction of adenosine and prostacyclin

Additional experiments were carried out in isolated hearts to evaluate the possibility that adenosine modulates prostacyclin release and vice versa.

The following experiments were performed to determine the effects of adenosine on prostacyclin release.

- Intracoronary infusion (1 min) of adenosine (10 nM, 0.1 μM, 1 μM) prior to and during infusion of indomethacin (5 μM, $n = 5$).
- Measurements of 6-keto-prostaglandin $F_{1\alpha}$ in samples of the coronary effluent collected prior to and after 10 min of ischemia in an isolated heart under control conditions (i.e., without treatment), during intracoronary infu-

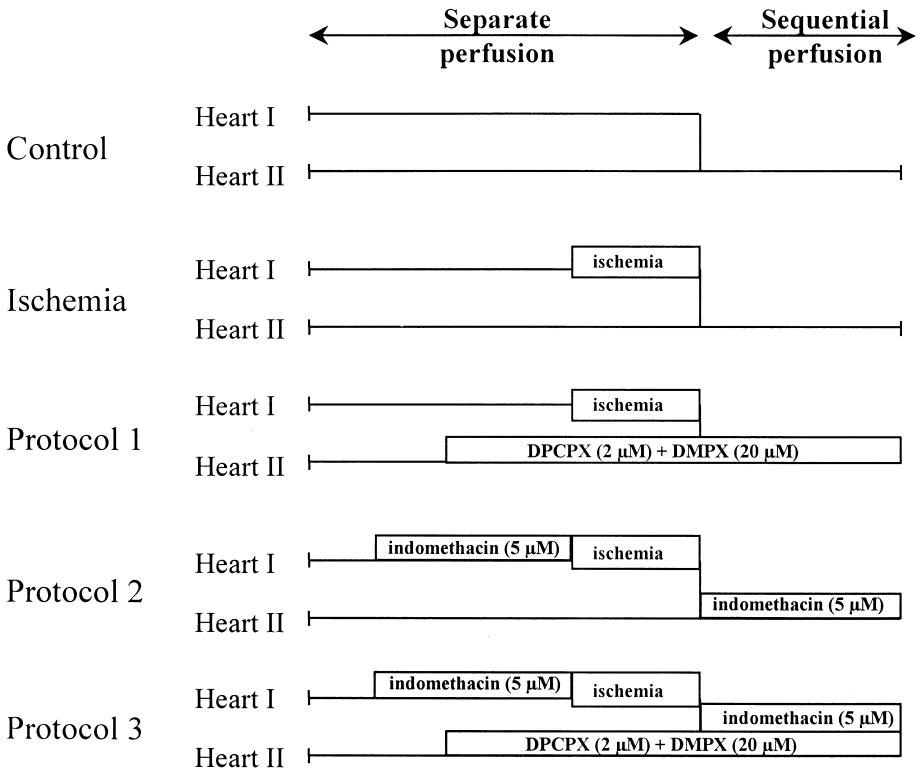

Fig. 1. Experimental protocol: the two hearts were separately perfused under control conditions (Control) and after 10 min ischemia in Heart I (Ischemia). The use of different protocols allowed investigation of the respective roles of adenosine and prostacyclin in post-ischemic vasoreactivity. In Protocol 1, the selective adenosine A_1 receptor antagonist (DPCPX, 2 μM) in combination with the adenosine A_2 receptor antagonist (DMPX, 20 μM) was infused into Heart II. In Protocol 2, indomethacin (5 μM) was infused intracoronarily into Hearts I and II. In Protocol 3, both the selective adenosine A_1 and A_2 receptor antagonists and indomethacin were administered.

sion of the adenosine A_1 receptor antagonist (DPCPX, 2 μM, $n = 5$), or during administration of the adenosine A_2 receptor antagonist (DMPX, 20 μM, $n = 5$).

The following experiments were performed to evaluate the modulation of adenosine release by prostacyclin.

- Intracoronary infusion (1 min) of the stable prostacyclin analogue iloprost (8.31 nM) prior to and during an infusion of the adenosine A_1 and A_2 receptor antagonists ($n = 3$).
- Measurements of adenosine overflow in samples of the coronary effluent collected during intracoronary infusion of iloprost ($n = 3$).
- Measurement of adenosine release after 10 min of global ischemia in an isolated heart, during/after administration of indomethacin infusion, in comparison to that determined in the control. The coronary effluent was collected after 10 min of global ischemia at the onset of reperfusion for 30 s, at 0, 0.5, 1, 1.5 and 2 min of reperfusion ($n = 5$).

2.3. Measurement of 6-keto-prostaglandin $F_{1\alpha}$: assays and sample processing

Immunoreactive 6-keto-prostaglandin $F_{1\alpha}$ was determined in unextracted samples as previously described (Schrör and Seidel, 1988). The separation of free and bound activity was performed by charcoal absorption. The detection limits (10% displacement of the radioactive tracer) were 7 pg/ml for 6-keto-prostaglandin $F_{1\alpha}$. The antibodies were raised in our laboratory and found not to cross-react (< 0.1% at the 50% displacement level) with a number of conventional prostaglandin standards (Schrör and Seidel, 1988).

2.4. Adenosine determination

Adenosine was determined by high-performance liquid chromatography (HPLC) measurements. For the analyses the coronary effluent was collected for 30 s prior to and after a 10-min period of global ischemia, at the onset of reperfusion, and at 0, 0.5, 1, 1.5 and 2 min of reperfusion. The effluent was instantly shock-frozen and stored at −70° until analysis. The samples were injected and analyzed by HPLC immediately after thawing. For each experiment, adenosine determination by HPLC began after reaching a stable baseline of the photodiode array detector. For the analyses a Nova-Pak reversed-phase column was used (150 × 3.9 mm, i.d., packed with 4-μm particles; Waters, Eschborn, Germany). The column temperature was maintained by a thermostat at 30°C (Model BFO-04, Peltier, TECHLAB, Germany). The mobile phase consisted of a 90/10 (v/v) mixture of ammonia acetate

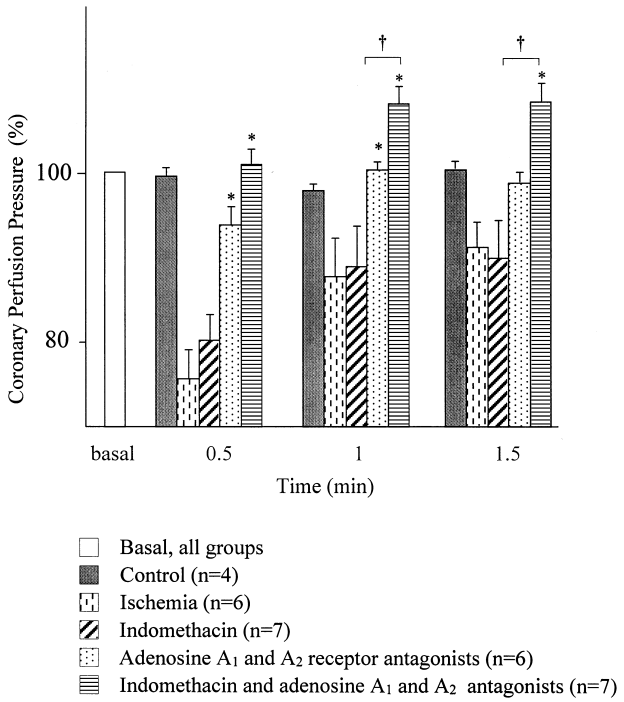

Fig. 2. Changes in coronary perfusion pressure in Heart II exposed to sequential perfusion under control conditions, after 10 min of ischemia in Heart I, and in the different drug-treated ischemic groups. *, $P < 0.05$ vs. ischemia without drug-treatment; †, $P < 0.05$.

(20 mM; pH 3.5 adjusted with 25% HCl) with methanol. Before use, the mobile phase was filtered through a 0.2-μm (pore size) filter membrane (Schleicher & Schuell, Dassel, Germany) under reduced pressure. The photodiode array detector was set at 260 nm for detecting adenosine. In addition, spectra were recorded throughout the entire chromatogram. The mobile phase was delivered at a flow rate of 1 ml/min. All chromatograms were recorded and the areas under the peaks of the respective analytes were integrated with a Millenium PDA, version 2.10 (Waters). Peaks representing adenosine in the chromatograms were identified on the basis of their retention times and the respective spectra. The detection limit of adenosine was 0.01 μM.

2.5. Ethics

The investigation conforms with the guide for care and use of laboratory animals published by the US National Institute of Health (NIH publication No. 85-23, revised 1985) and 'The Guiding Principles for Research Involving Animals and Human Beings'.

2.6. Statistical analysis of data

Unless otherwise indicated, results are expressed as means ± S.E.M. for n determinations. Statistical analyses were performed by Kruskal–Wallis analysis of variance (ANOVA), followed by the Mann–Whitney U-test at a level of significance of $P < 0.05$.

2.7. Drugs

Adenosine and indomethacin were purchased from Sigma (Deisenhofen, Germany). DPCPX and DMPX were obtained from Research Biochemicals International (RBI; Cologne, Germany). Iloprost was obtained from Schering (Berlin).

3. Results

3.1. Hemodynamic changes during serial perfusion in sequentially perfused second hearts, after 10 min of global ischemia of the first heart

In both groups, left ventricular contractile parameters as well as heart rate and coronary perfusion pressure were similar at baseline. No significant changes of these parameters were observed in the second heart during sequential perfusion in the control group ($n = 4$) (Figs. 2 and 3). However, when sequential perfusion was performed after 10 min of global ischemia of the first heart, a pronounced decrease in coronary perfusion pressure (−23%) was observed in Heart II ($n = 6$, Fig. 2). This vasodilation was reversible within 5 min. In addition, a decrease in left ventricular contractile parameters occurred in Heart II

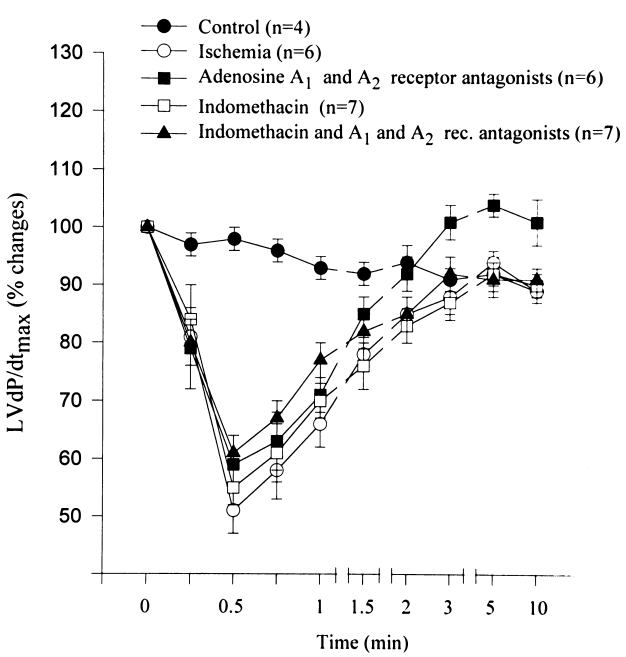

Fig. 3. Time plot of changes in $LVdP/dt_{max}$, representative for changes in contractile parameters in Heart II during sequential perfusion under control conditions and after 10 min of global ischemia with/without drug-treatment. $LVdP/dt_{max}$ was significantly different ($P < 0.05$) from control in all groups subjected to ischemia between 0.5 and 1 min after the onset of sequential perfusion.

Table 1
Basal left ventricular hemodynamic indexes as well as heart rate and coronary perfusion pressure of heart II immediately prior to sequential perfusion in the different groups. Values are means ± S.E.M.

Group	Heart rate (beats/min)	LVP (mm Hg)	LVdP/dt_{max} (mm Hg/s)	LVdP/dt_{min} (mm Hg/s)	Coronary perfusion pressure (cm H_2O)
Control ($n=4$)	216 ± 3	72 ± 3	1566 ± 122	−1306 ± 107	88 ± 7
Ischemia ($n=6$)	218 ± 8	69 ± 3	1443 ± 70	−1250 ± 54	83 ± 5
Adenosine A_1 and A_2 receptor antagonists ($n=6$)	214 ± 5	73 ± 4	1453 ± 58	−1120 ± 44	94 ± 2
Indomethacin ($n=7$)	216 ± 9	75 ± 3	1559 ± 81	−1209 ± 44	83 ± 4
Adenosine A_1 and A_2 receptor antagonists + indomethacin ($n=7$)	219 ± 6	70 ± 5	1491 ± 159	−1174 ± 96	90 ± 1

immediately at the onset of sequential perfusion. LVdP/dt_{max}, representative of changes in contractile parameters, decreased by 49% (Fig. 3). Heart rate at baseline was 216 ± 3 beats/min and did not change significantly during the experiments (Table 1).

3.2. Effects of DPCPX and DMPX on vasorelaxation in sequentially perfused second hearts after 10 min of ischemia in the first heart (Protocol 1)

Dose-finding experiments revealed that DPCPX (2 μM) infused in combination with DMPX (20 μM) completely inhibited all vasodilating effects of an intracoronary infusion of exogenous adenosine up to 1 μM. Infusion of DPCPX and DMPX into Heart II under basal conditions induced significant changes neither in coronary perfusion pressure (increase by 4 ± 3%) nor in the contractile parameters. During sequential perfusion after 10 min of ischemia in Heart I, the selective adenosine receptor antagonists inhibited the maximum decrease in coronary perfusion pressure in Heart II by 74% (Fig. 2). No significant changes in contractile parameters were observed, however (Fig. 3).

3.3. Effects of prostacyclin synthesis inhibition on vasodilation observed in sequentially perfused hearts after 10 min of ischemia in the first heart (Protocol 2)

To investigate the influence of post-ischemic prostacyclin release on coronary circulation in the second heart,

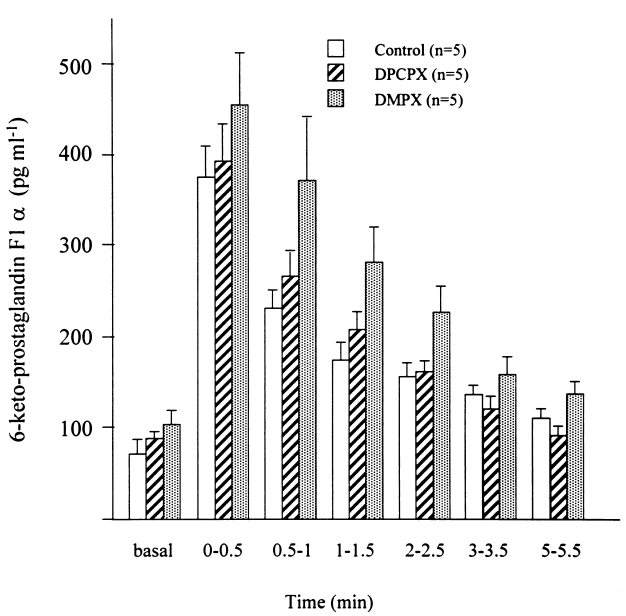

Fig. 4. Time plot of the release of 6-keto-prostaglandin $F_{1\alpha}$ prior to (basal) and after 10 min of global ischemia in an isolated heart during reperfusion under control conditions (Control), and during infusion of the adenosine A_1 receptor antagonist (DPCPX) or the adenosine A_2 receptor antagonist (DMPX).

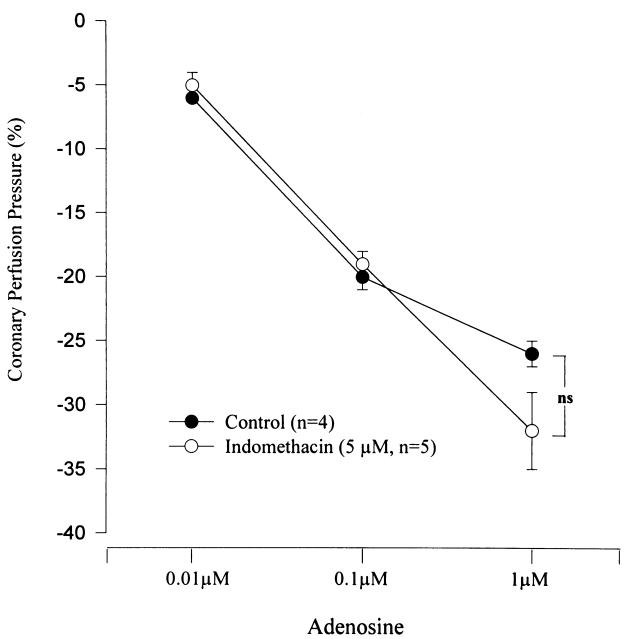

Fig. 5. Concentration/response curves of changes in coronary perfusion pressure in response to adenosine prior to (Control) and during intracoronary infusion of indomethacin. Values are means ± S.E.M. ns, Not significant.

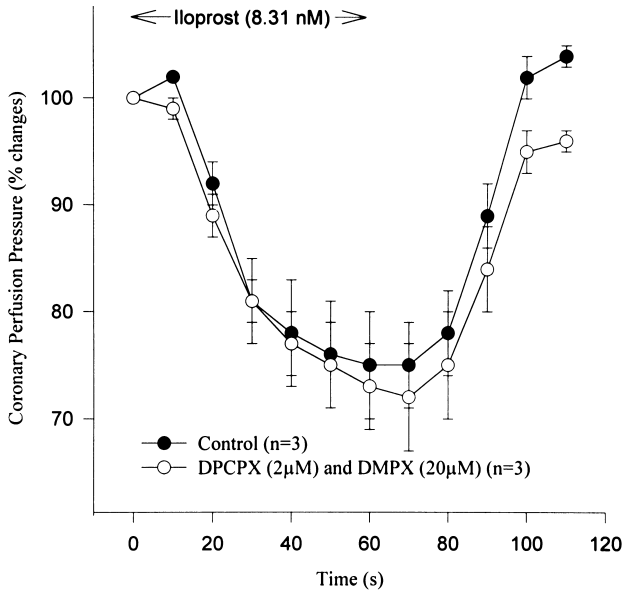

Fig. 6. Effect of intracoronary infusion of the stable prostacyclin analogue iloprost under control conditions (Control) and during infusion of the selective adenosine A_1 receptor antagonist (DPCPX) in combination with the adenosine A_2 receptor antagonist (DMPX).

Heart I and Heart II were pretreated with a prostacyclin synthesis inhibitor ($n = 7$). Under basal conditions, infusion of indomethacin (5 μM) induced merely a slight and not significant increase in coronary perfusion pressure in Hearts I and II. At the onset of serial perfusion after 10 min of global ischemia in Heart I, this cyclooxygenase inhibitor did not significantly influence the decrease in coronary perfusion pressure in Heart II (Fig. 2). Furthermore, no relevant changes in contractile parameters occurred (Fig. 3).

3.4. Combined effect of adenosine receptor antagonists and indomethacin (Protocol 3)

In the presence of both indomethacin and the selective adenosine A_1- and A_2-receptor antagonists, complete inhibition of vasodilation in the second heart during sequential perfusion after 10 min of ischemia in the first heart was observed (Fig. 2). Moreover, after 60 s of sequential perfusion, a moderate but significant ($P < 0.05$) increase in coronary perfusion pressure was also detected. Left ventricular contractile parameters did not statistically differ from those of the other groups (Fig. 3).

3.5. Lack of involvement of prostacyclin in adenosine-mediated vasodilation

The release of 6-keto-prostaglandin $F_{1\alpha}$ into the coronary effluent after 10 min of global ischemia in an isolated heart was not modulated by intracoronary infusion of the adenosine A_1 or A_2 receptor antagonists DPCPX and DMPX (Fig. 4). Furthermore, in a different series of experiments, the direct effects of adenosine on prostacyclin production were examined in isolated perfused hearts. Adenosine infused intracoronarily into isolated hearts in three different concentrations (0.01, 0.1 and 1 μM) induced a decrease in coronary perfusion pressure by $6 \pm 0\%$, $20 \pm 1\%$ and $24 \pm 1\%$, respectively (Fig. 5). No significant differences in the decrease in adenosine-induced coronary perfusion pressure were observed during intracoronary infusion of indomethacin. This implies that prostacyclin is not involved in adenosine-mediated vasodilation.

3.6. Prostacyclin inhibits adenosine release

To exclude the possibility that adenosine contributes to the vascular effects of prostacyclin, the stable prostacyclin analogue, iloprost (8.31 nM), was intracoronarily infused into an isolated heart prior to and during administration of adenosine A_1 and A_2 receptor antagonists ($n = 3$). As illustrated in Fig. 6, the vascular response to iloprost was not significantly different in the absence or presence of the adenosine receptor antagonists. In addition, determination of adenosine by HPLC in the coronary effluent collected during intracoronary infusion of iloprost revealed that this prostacyclin analogue did not induce a release of adenosine (data not shown).

Determination of adenosine in the coronary effluent collected after 10 min of global ischemia in an isolated heart during reperfusion revealed an increase in adenosine concentration which peaked during the first 30 s; the concentration then decreased with time (Fig. 7). Interest-

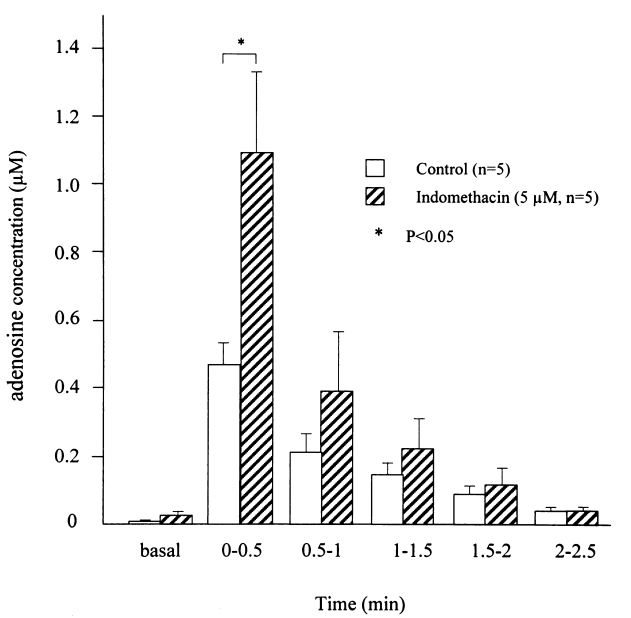

Fig. 7. Time plot of the release of adenosine prior to (basal) and after 10 min of global ischemia in an isolated heart during reperfusion under control conditions (Control) and during infusion of indomethacin.

ingly, this adenosine release is significantly enhanced when the heart is treated with an indomethacin infusion. These results imply that prostacyclin has an inhibitory effect on adenosine release after myocardial ischemia.

4. Discussion

We have recently shown that cardiodepressant mediators are released after myocardial ischemia from an isolated heart during reperfusion which induced a pronounced decrease in contractility in a sequentially perfused, non-ischemic second heart used as a bioassay (Felix et al., 1997; Stangl et al., 1997). These studies did not further investigate the mechanism of the simultaneously observed, post-ischemic vasodilation. Since vasodilation occurs in the sequentially perfused recipient heart which had not been rendered ischemic, it was possible to ascertain mediator-induced changes in coronary vasomotion, independently of confounding metabolic, myogenic and neurogenic factors due to ischemia itself. Whereas the role of NO as vasodilator in the early phase of reactive hyperemia appears to be universally accepted, conflicting results have been published with respect to interaction of adenosine and prostacyclin in vasoregulation after myocardial ischemia. Furthermore, species differences have been reported in terms of adenosine formation in the ischemic myocardium (Van Belle et al., 1985)

The objective of this study was to investigate the interaction of adenosine and prostacyclin in post-ischemic vasodilation. Post-ischemic vasodilation in our model was primarily due to an adenosine release. Prostacyclin — also released into the coronary effluent after 10 min of global ischemia — apparently does not play a predominant role. These results are in accordance to data obtained by Gryglewski et al. (1995b). Interestingly, our results suggest that prostacyclin inhibits adenosine release after myocardial ischemia: during indomethacin infusion at a concentration sufficient to prevent ischemia-induced formation of prostacyclin, we observed augmented adenosine overflow into the coronary effluent after 10 min of global ischemia. This mechanism may explain the phenomenon observed in our model that indomethacin alone was without effect on coronary vasomotion after myocardial ischemia. Indeed, the increased adenosine release compensates for the reduced prostacyclin formation. However, when the effects of adenosine were blocked by selective receptor antagonists, prostacyclin likely contributed to the post-ischemic increase in coronary flow. This implies that prostacyclin represents an additional compensatory vasodilatory mechanism which increases in significance when the vascular action of adenosine is prevented (Fig. 2). The underlying mechanism by which prostacyclin inhibits adenosine release remains to be elucidated.

It must be emphasized that NO — implicated essentially in the early phase of post-ischemic vasodilation (Park et al., 1992) and in reactive hyperemia which follows brief coronary occlusions (Hansen and Haunso, 1995) — was not assessed in our experimental setup of the double heart model in view of the short half life of this autacoid. Nevertheless, to exclude the possibility that formation of NO in the donor or recipient heart may contribute to the vasodilation observed in our model, we performed additional experiments ($n = 5$) in which the NO synthase inhibitor N^{ω}-nitro-L-arginine (100 μM) was infused into both hearts starting 40 min prior to sequential perfusion. As anticipated, this prolonged administration of the NO synthase inhibitor was ineffective in modulating vasodilation in the second heart during sequential perfusion after 10 min of ischemia in the first heart (data not shown).

There are contradictory reports concerning the interactions of adenosine and prostacyclin in regulating coronary vasodilation. Adenosine has been reported to increase the synthesis of cardiac prostaglandins: primarily, prostacyclin from the isolated rabbit heart (Ciabattoni and Wennmalm, 1985; Karwatowska-Prokopczuk et al., 1988). Felsch et al. (1994) proposed that, in the isolated guinea pig heart, both highly selective adenosine A_1 and A_2 receptor agonists were capable of augmenting prostacyclin formation in the heart. On the other hand, Cano et al. (1992) postulated that adenosine inhibits cardiac prostacyclin production in the rabbit heart. Conversely, Blass et al. (1980) have suggested that the coronary vasodilator effects of prostacyclin in the isolated perfused rabbit heart are at least partially due to the release of adenosine. In contrast to this report, a reduction has been observed in adenosine release after application of prostacyclin in the isolated rat heart (Schrör et al., 1980). These results are in accordance with the findings of the present study and imply that post-ischemically released prostacyclin reduces adenosine overflow. Since indomethacin infusion with consecutive increased adenosine overflow was not accompanied by changes in vasomotion in the sequentially perfused second heart, it may be hypothesized that coronary active prostaglandins such as prostacyclin may play a more complex role in coronary flow regulation through a feedback mechanism, rather than through direct vasodilator effects.

Our investigations failed to document a modulating role of adenosine on prostacyclin formation. In addition to species differences, one possible explanation for these conflicting results may be different methodological setups. A noteworthy feature of our experimental setup is that only mediator-induced vasodilation after myocardial ischemia was investigated in a recipient heart, and that metabolic, myogenic and neurogenic effects due to ischemia itself were excluded. Furthermore, changes in vasoreactivity secondary to ischemia-induced functional and/or structural abnormalities — such as morphological alterations of the capillaries, depression of vascular smooth muscle reactivity, interstitial edema, and endothelial dysfunction — were omitted (Triana and Bolli, 1991). Fi-

nally, in this buffer-perfused heart preparation the role of blood cells (and therefore the potential effects of vascular plugging or blood-cell-mediated chemical reactions) is eliminated. The present protocol was designed to avoid the confounding influence of these factors on evaluation rendered of the role of adenosine and prostacyclin in mediating post-ischemic vasodilation.

In conclusion, the present study has three primary implications. (1) It further corroborates the concept that, independent of metabolic, myogenic and neurogenic factors, post-ischemic vasodilation is chiefly due to adenosine release; (2) it implies that prostacyclin released after ischemia partially inhibits adenosine release; and (3) it indicates that the action of prostacyclin may represent compensatory vasodilator mechanisms when vascular action of adenosine is prevented.

Acknowledgements

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