Molecular and Cellular Biochemistry **88**: 113–121, 1989.

Invited Paper

Significance of myocardial eicosanoid production

Marc van Bilsen[1], Wim Engels[2], Ger J. van der Vusse[1] and Robert S. Reneman[1]
Departments of Physiology[1] and Medical Microbiology[2], University of Limburg, Maastricht, the Netherlands

Accepted 28 December 1988

Key words: eicosanoids, arachidonic acid, ischemia, reperfusion, myocardium

Summary

The precise role of eicosanoids in the development of myocardial injury during ischemia and reperfusion is still a matter of debate. Enhanced local production of these bioactive compounds appears to be a common response to tissue injury. Most likely, the cardiac tissue has the capacity to generate prostaglandins, thromboxanes as well as leukotrienes. Prostacyclin (PGI_2) is the major eicosanoid produced by the jeopardized myocardium. In addition, at sites of tissue injury activation of platelets and infiltrating leukocytes results in the formation of considerable amounts of thromboxanes and leukotrienes. The production of eicosanoids requires prior release of arachidonic acid (AA) from phospholipids. Both ischemia and reperfusion are associated with a rise in the tissue level of AA. The absence of a proportional relationship between the tissue level of AA and the amounts of PGI_2 produced suggests that the sites of AA accumulation and PGI_2 formation are different. It is conceivable that AA accumulation is mainly confined to myocytes, whereas the capacity to synthesize PGI_2 mainly resides in vascular cells. Both beneficial and detrimental effects of eicosanoids on cardiac tissue have been described. Prostaglandins act as vasodilators. Besides, some of the prostaglandins, especially PGI_2, are thought to possess cyto-protective properties. Thromboxanes and leukotrienes may impede blood supply by increasing smooth muscle tone. Besides, leukotrienes augment vascular permeability. Experimental studies, designed to evaluate the effect of pharmacological agents, like PGI_2-analogues and lipoxygenase and cyclo-oxygenase inhibitors, indicate that eicosanoids influence the outcome of myocardial injury. However, the delineation of the physiological significance of the locally produced eicosanoids is complicated by such factors as the wide variety of AA derivatives produced and the dose-dependency of their effects.

Introduction

The enzymatic oxidation of fatty acids with a 20-carbon structure, i.e. eicosatrienoic, eicosatetraenoic and eicosapentaenoic acid, leads to a variety of products, commonly referred to as 'eicosanoids'. The eicosanoids represent a family of biologically very potent and versatile compounds, which are thought to be of importance as modulators of a multitude of (patho)physiological processes, like thrombosis, inflammation, immune response, tissue perfusion and atherosclerotic diseases. Recent findings indicate that myocardial ischemia and reperfusion can elicit a local generation of eicosanoids. Besides, at sites of myocardial injury eicosanoids will be produced by platelets and leukocytes. The enhanced local production and versatile bioactive properties of the arachidonic acid (AA) derivatives suggests that these substances play a role as mediators and/or modulators of myocardial injury during ischemia and reperfusion. Nevertheless, it is still obscure to which extent and in which manner

these locally produced eicosanoids affect the outcome of a (transiently) ischemic insult. Studies directed to elucidate the role of eicosanoids in myocardial ischemia and reperfusion are complicated by such problems as

(a) methodological aspects of their determination,
(b) short half-lives,
(c) the dose-dependency of their effects,
(d) mutual interaction of individual eicosanoids,
(e) the large number of cell-types involved, each producing a different spectrum of eicosanoids, and
(f) the limited specificity of the pharmaca used to modulate eicosanoid formation and function.

The arachidonic acid cascade

Since in mammalian species eicosatetraenoic acid, i.e. AA, is by far the most important substrate for the enzymes involved, the entire process of eicosanoid formation is also often referred to as the 'arachidonic acid cascade'. In this cascade the oxygenation of AA can either be catalyzed by lipoxygenases, which leads to the formation of hydroperoxide-derivatives (HPETE's), or by cyclo-oxygenase, which leads to the formation of endoperoxide-derivatives, such as PGG_2 and PGH_2 (Fig. 1). Recently, a third pathway, the so-called epoxygenase pathway, for the metabolism of AA has been discovered in renal tissue [1]. In this metabolic route AA is converted into, among others, epoxides and HETE's through cytochrome P-450 dependent mono-oxygenases. It is unknown whether the enzyme system is also involved in the production of eicosanoids in the myocardium.

In the lipoxygenase pathway the HPETE's are converted to their corresponding hydroxy acids (HETE's). Moreover, 5-HPETE can also be transformed into leukotriene A_4 (LTA_4). LTA_4, in turn, can be converted to LTB_4 or the peptide leukotriene LTC_4. In the latter case the tripeptide glutathione (GSH) is coupled to LTA_4, a reaction catalyzed by glutathione-S-transferase. The subsequent elimination of the glutamic acid and glycine moieties from LTC_4 yields LTD_4 and LTE_4, respectively. In the cyclo-oxygenase pathway the enzy-

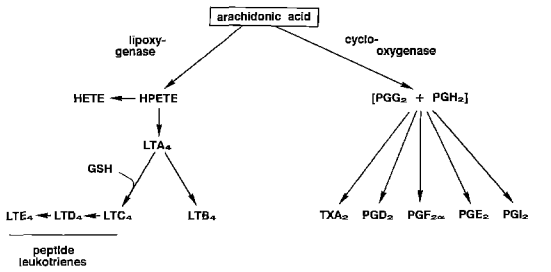

Fig. 1. The arachidonic acid cascade. In this simplified scheme only the major pathways of eicosanoid formation are shown. HPETE and HETE indicate hydroperoxy and hydroxy eicosatetraenoic acid, respectively. Other abbreviations are explained in the text.

matic conversion of the endoperoxide PGH_2 leads to two classes of products, the thromboxanes (TX's) and the prostaglandins (PG's). Thromboxane synthase catalyzes the conversion of PGH_2 into TXA_2. Various transformations, involving isomerases and reductases, lead to the synthesis of the different PG's, among which prostacyclin or PGI_2 (Fig. 1).

Both TXA_2 and PGI_2 are chemically unstable compounds and are non-enzymatically converted into their biologically less active or inactive stable analogues, TXB_2 and 6-keto-$PGF_{1\alpha}$, respectively. This conversion occurs rapidly. Half lifes of only 30 seconds and 5 to 10 minutes have been reported for TXA_2 and PGI_2, respectively [2]. Moreover, in the circulation both products are rapidly metabolized by various enzymes. The consequence of the chemical conversion is that the sites of formation and action of the eicosanoids most likely coincide. In other words, the eicosanoids are autacoids, i.e. potent modulators and/or mediators of cellular function, which are generated and act locally.

Cardiac eicosanoid production

The heart is composed of various cell types. On a volume base by far the largest part of the tissue is accounted for by myocytes. With respect to the cardiac eicosanoid generating capacity other cell types, like endothelial cells, smooth muscle cells and fibroblasts, are probably of more importance. Using ^{14}C-archidonate and a differential labeling

technique Hsueh and Needleman [3] were able to demonstrate that in the isolated rabbit heart the vasculature is the principal site of PG formation. *In vivo* blood cells may contribute to cardiac eicosanoid production. Substantial amounts of TX's may be formed in response to platelet activation. LT's are produced by leukocytes, when activated. Notably, in response to certain stimuli, like the calcium-paradox or the calcium ionophore A23187, LT's are released from isolated, Krebs-Henseleit perfused, hearts [4, 5] indicating that the cardiac tissue is able to synthetize LT's.

It is unclear whether eicosanoids are produced under normoxic conditions and, if so, whether this is of physiological importance. Under resting conditions 6-keto-$PGF_{1\alpha}$ levels were found to be higher in the coronary sinus than in the aorta of the open-chest dog. No such difference was observed for TXB_2. These findings suggest that under normoxic conditions the *in vivo* heart releases PGI_2, but not TXA_2 [6]. The normoxically perfused, isolated heart produces PGI_2 [7–9]. Besides, PGE_2 and $PGF_{2\alpha}$ are synthetized, although in appreciably lower quantities [7, 8]. De Deckere [10] reported that the concentrations of PGI_2 released from normoxically perfused hearts are in the same range as those that elicit a vasodilatory effect.

Ischemia and reperfusion are potent stimuli to activate the AA cascade. Although questioned by some investigators [11–13], it is generally believed that coronary artery occlusion leads to enhanced production of PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2 [14–17]. Restoration of flow may even enhance cardiac eicosanoid production [8, 18].

After application of exogenous AA adult myocytes were able to synthetize PG's, like PGI_2, $PGF_{2\alpha}$, PGD_2 and PGE_2 [19, 20]. The main products generated by cultured endothelial cells are PGI_2, PGE_2, and $PGF_{2\alpha}$. The ratio between the amounts of PGI_2, PGE_2 and $PGF_{2\alpha}$ released may depend on the origin of the endothelial cells (species, organ, arterial or venular, vessel-size) [21–23]. PGE_2 was reported to be the predominant eicosanoid species released by smooth muscle cells and fibroblasts [24]. The ability of endothelial cells, smooth muscle cells and fibroblasts to produce TXA_2 or LT's, if any, is low [19, 22, 24, 25].

Regulation of cardiac eicosanoid production

In theory, the synthesis of eicosanoids depends on the activity of the enzymes and the availability of substrate and co-factors (molecular oxygen, peroxides, glutathione, calcium). It has been shown that the rates of eicosanoid formation, by for instance endothelial cells or platelets, are far below the reported V_{max} values of the different enzymes involved. So, the enzyme concentration appears not to be rate-limiting. Furthermore, the oxygenases have a high affinity for O_2 (K_m value of about 5 μM). Therefore, only under conditions of very severe ischemia the concentration of this essential co-factor will put restraints on eicosanoid synthesis [26]. Furthermore, it has been established that 5′-lipoxygenase is a calcium-dependent enzyme [2]. Peroxides are known to modulate cyclo-oxygenase activity. Low concentrations appear to be required, whereas higher concentrations inactivate the enzyme [2]. GSH is required as co-substrate for the synthesis of peptide LT's. It is conceivable that the ischemia/reperfusion-induced generation of reactive oxygen species and alterations in the tissue glutathione level affect eicosanoid production.

AA is the main substrate for the cyclo-oxygenase and lipoxygenases. The cardiac content as well as the serum level of AA is very low, i.e. in the order of 2 nmol/g wet weight and 3 nmol/ml of plasma, respectively [27]. However, in the tissue AA is amply present in bound form. The majority of bound AA is incorporated in phospholipids, important constituents of cellular membranes. The amount of AA esterified to phospholipids amounts to about 12 μmol/g wet weight [28]. This implies that less than 0.02% of AA is present in the unesterified form. This empirical finding underlines the notion that the endogenous production of eicosanoids depends on the enzymatic release of AA from phospholipids.

The phospholipid pool is in dynamic equilibrium, i.e. continuously subjected to deacylation and reacylation of the fatty acyl moieties. Accordingly, enhanced release of AA from the phospholipid pool could either result from an activation of phospholipases and/or impairment of the incorporation of fatty acids into phospholipids. Increased

hydrolysis of phosphatidylcholine by phospholipase A_2 has been suggested to be the major pathway of AA release. In addition, enhanced activity of phosphatidylinositol specific phospholipase C in combination with diglyceridase activity contributes to the release of AA. Possibly, the preferred pathway of AA release varies for each cell-type.

Reacylation of phospholipids is most likely regulated at the level of fatty acid activation, a reaction catalyzed by acylCoA synthetase. This reaction is ATP dependent and is inhibited by AMP and adenosine. About the relevance of a reduced reacylation of AA into phospholipids in the formation of eicosanoids can only be speculated. This mechanism might be operative during ischemia since under these conditions tissue levels of ATP decrease and AMP and adenosine increase [29].

Influx of calcium is currently considered the most likely mechanism to activate phospholipase activity and, hence, AA release. This notion is substantiated by observations demonstrating that PG synthesis is stimulated by the calcium ionophore A23187 and is inhibited at low calcium concentrations or in the presence of calcium channel blockers [30].

It has been stated by various investigators that the availability of substrate, i.e. AA, is the rate limiting step in the formation of eicosanoids from tissues [31]. During ischemia the tissue level of AA rises. Reperfusion does not reverse this process, but promotes further accumulation of AA [29]. If the rate of eicosanoid synthesis is determined by the availability of AA, a relation between AA accumulation and eicosanoid formation might be anticipated. In line with this notion is the observation that the cumulative release of 6-keto-$PGF_{1\alpha}$ during reperfusion of isolated rat hearts is related to the tissue content of AA at the end of the preceding ischemic period (Fig. 2). In a similar model Karmazyn and coworkers [32] also observed an enhanced release of 6-keto-$PGF_{1\alpha}$ upon reperfusion. In their study the amount released did not depend on the time duration of the preceding ischemic period. In addition, 6-keto-$PGF_{1\alpha}$ release already occurred after periods of ischemia that are not likely to elicit a rise in the tissue content of AA [29, 33].

Following ischemia the release of 6-keto-$PGF_{1\alpha}$

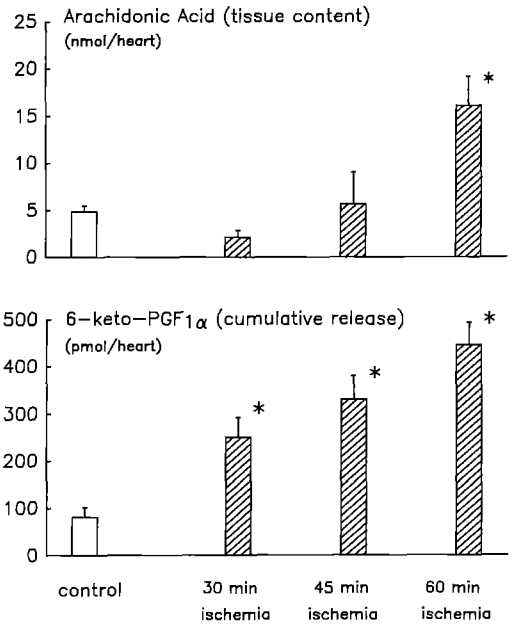

Fig. 2. Tissue content of arachidonic acid of normoxically perfused isolated rat hearts and hearts subjected to 30, 45 and 60 min of global ischemia (A) and the cumulative release of 6-keto-$PGF_{1\alpha}$ into the coronary effluent during the subsequent 30 min of (re)perfusion (B). Means ± SD. * $p<0.05$ vs control.

is of transient nature, whereas AA continues to accumulate during the entire reperfusion phase (Fig. 3). Moreover, following 60 min of ischemia the amount of 6-keto-$PGF_{1\alpha}$ released during reperfusion represents less than 0.5% of the AA content of reperfused hearts. The transient character of PGI_2 synthesis has been ascribed to peroxide-induced self-deactivation of the cyclo-oxygenase enzyme [26]. Alternatively, it is feasible that either the release of PGI_2 is not solely determined by the availability of AA or the sites of AA accumulation and eicosanoid formation do not coincide [5, 9]. This notion is further supported by the observations that, as compared with transient ischemia, induction of the calcium paradox and administration of A23187 resulted in smaller increases in the tissue AA content, but a more pronounced release of 6-keto-$PGF_{1\alpha}$ from isolated rat hearts [5].

Several other findings also argue against a direct relationship between AA availability and eicosanoid production. Otani and colleagues [18] demonstrated that reperfusion following global ischemia

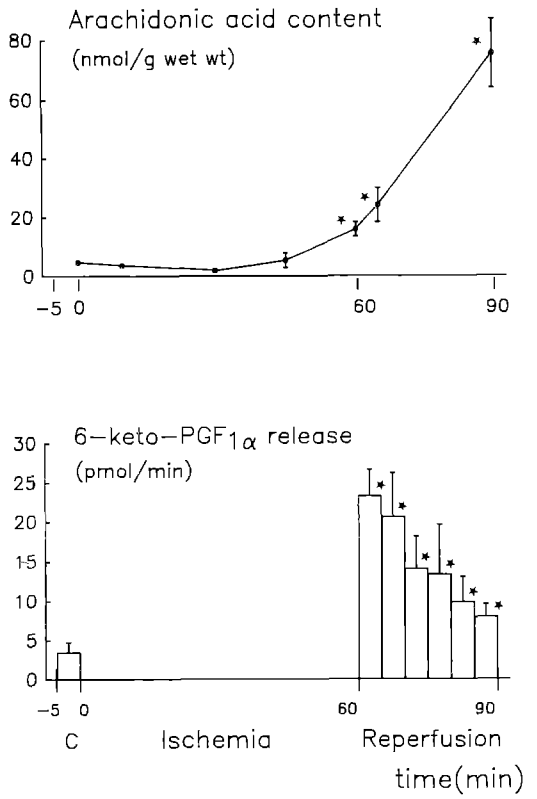

Fig. 3. Time course of changes in the tissue content of arachidonic acid (A) and the release of 6-keto-$PGF_{1\alpha}$ (B) during 60 min of ischemia followed by 30 min of reperfusion of isolated rat hearts. Means ± SD. *$p<0.05$ vs preischemic value.

of pig hearts was associated with a substantial decrease of the cardiac phospholipid content (over 20%) and release of 6-keto-$PGF_{1\alpha}$. Administration of both mepacrine and superoxide dismutase (SOD) plus catalase prevented the degradation of phospholipids. However, mepacrine reduced 6-keto-$PGF_{1\alpha}$ release, whereas SOD plus catalase increased 6-keto-$PGF_{1\alpha}$ release. Finally, studies using isolated hearts prelabeled with ^{14}C-AA, indicate that the administration of bradykinin results in a tight coupling between AA release from phospholipids and prostaglandin formation [34, 35]. These findings suggest that this agent stimulates the myocardial production of eicosanoids by triggering the release of AA from a phospholipid pool, closely linked to the AA converting enzymes. Under these circumstances the formation of eicosanoids might also be rather insensitive to the overall tissue AA level.

Do eicosanoids play a role in myocardial ischemia and reperfusion?

Reviewing the evidence for the contribution of LT's as mediators in ischemia and shock, Lefer [36] formulated four criteria which should be met by any substance in order to be considered as a mediator of a pathophysiological state. First of all, such a substance should exert one or more pathophysiological effects. Second, its production would have to increase during the development of the pathophysiological state. Third, if exogenously applied it should mimic some of the effects of the disease state. Finally, inhibition of the mediator should diminish the disease state.

Indeed, the production of several potentially harmful eicosanoids is enhanced during ischemia and/or reperfusion. Investigations employing (a) cyclo-oxygenase or lipoxgenase inhibitors or (b) thromboxane synthase inhibitors or thromboxane receptor antagonists demonstrate that these compounds are of therapeutic use in the setting of myocardial ischemia and reperfusion. In addition, the administration of eicosanoids, like TXA_2, LTC_4 or LTD_4, known to exert deleterious effects, evokes pathophysiological phenomena in the heart [37]. These findings suggest that these eicosanoids are true mediators of myocardial injury.

Studies on hormones indicate that generally these compounds are biologically active at concentrations in the same order of magnitude as the association constant of these ligands and their receptors. This would imply that PG's are effective at concentrations in the order of 10^{-9} to 10^{-8} mol/litre. On the basis of these arguments Schroer [38] infered that dose-dependent biological effects of PG's and TX's occur at concentrations in the nanomolar range. The corollary of this notion is (a) that local concentrations should reach this level in order to become effective and (b) that studies in which these substances are found to be effective only at much higher concentrations should be interpreted with care.

Leukotrienes

LTB_4 is a very potent chemotactic, whereas the peptide LT's are known for their vasoconstrictive effects and their ability to increase vascular permeability [36]. Vasoconstriction as well as edema formation might lead to a further impedement of tissue perfusion during ischemia and/or no-reflow during reperfusion.

Circumstantial evidence indicates that LT's are no virtual mediators of cardiac cell death. Increased release of LT's is only observed after 24 hours of coronary artery occlusion, whereas the onset of irreversible cell damage takes place within a few hours. LT release parallels the infiltration of polymorphonuclear neutrophils (PMN's) in the infarcted area and, hence, the PMN's are considered the main source of the LT's produced. It has been proposed that the production of LT's by PMN's is related to the late arrhythmias and to infarct expansion [39]. Indeed, various studies have shown that lipoxygenase inhibitors are able to reduce infarct size [40, 41]. Accordingly, lipoxygenase inhibitors may attenuate the infiltration and subsequent activation of PMN's and, in this way, reduce the production of reactive oxygen species and the release of proteolytic enzymes in the infarcted area. It should be noted, that beneficial effects of lipoxygenase inhibitors have been reported, either preceding PMN infiltration or being unrelated to the number of PMN's infiltrating the infarcted area [41]. However, interpretation of studies with lipoxygenase inhibitors is complicated by the fact that the specificity of many of these drugs is limited. Most of them also act as free radical scavengers [40].

In cultures of myocardial cells it was found that during hypoxia creatine kinase release, a marker for cell necrosis, preceded the release of LTC_4. Furthermore, the addition of LTC_4 to the culture medium did not evoke enzyme release. Accordingly, release of LT's was not considered a mediator, but only a result of cell injury [42].

Thromboxanes

TXA_2 is a strong vasoconstrictor and has pro-aggregatory effects. In this way platelet derived TX's might aggrevate the degree of ischemia by further reducing blood supply. Both TX synthase inhibitors and TX receptor antagonists have been shown to reduce enzyme release during myocardial ischemia [43, 44] and to preserve the myocardial adenine nucleotide content in reperfused dog hearts [45]. Often TX synthase inhibitors also augment PGI_2 and PGE_2 production. Hence, it is hard to delineate whether the observed effects are actually a result of the prevention of TXA_2 synthesis or the induction of PG synthesis. TX receptor antagonists do not affect TXA_2 levels and therefore may provide more direct insight into the pathophysiological role of TX's.

Prostaglandins

Although not equally potent, in general, the PG's induce vasodilatation and prevent platelet aggregation. Conflicting results have been reported regarding the inotropic and electrophysiological effects of PG's (reviewed by Karmazyn and Dhalla [46]). In addition, some of the PG's (PGE_2, PGI_2) are said to possess 'cyto-protective' properties. This term refers to the beneficial effects of eicosanoids on (transiently) ischemic cardiac tissue, that appear not to be related to systemic effects, tissue perfusion and alterations in the inotropic or chronotropic state. PGI_2 is not only the quantitatively most important eicosanoid generated by the myocardium, but also the most potent representative of the prostaglandin family.

Upon reperfusion the 6-keto-$PGF_{1\alpha}$ concentration in the coronary effluent of isolated working rat hearts peaks and reaches values averaging 1.5 nmol/litre [9]. Therefore the amounts of PGI_2 produced are in the order of magnitude as the association constant of the prostacyclin receptor [38] and, as a consequence, will likely affect cellular processes.

Numerous studies with *in vivo* heart models have provided evidence that infusion of PGI_2 or its analogue iloprost affords substantial protection to the ischemic and reperfused myocardium [47–51]. In the *in vivo* model various factors may contribute to

the protective effects of PGI_2 or iloprost, such as a reduction of mean arterial pressure (i.e. reduction of workload), coronary vasodilatation, inhibition of platelet aggregation and/or leukocyte infiltration, and the so-called cyto-protective effects.

In *ex vivo* heart models both beneficial and detrimental effects of PGI_2 have been reported. It should be noted that in these models effects of PGI_2 on peripheral resistance and platelet and leukocyte function are irrelevant. Araki and Lefer [52] found a reduction of enzyme release and improved post-ischemic recovery of isolated cat hearts treated with PGI_2. Other investigators [53, 54] reported no or only modest protective effects. Karmazyn [8, 32] and Moffat [55] showed that PGI_2 exerts deleterious effects, as evidenced by a decrease of post-ischemic function and an improved functional recovery and reduction of enzyme release when isolated rat hearts are perfused in the presence of PG synthase inhibitors. Moreover, the beneficial effect of these inhibitors was reversed by the concomitant administration of PGI_2.

The modulation of adenylate cyclase activity via a PGI_2 receptor likely represents an important mechanism by which this substance exerts its bioactive effects. The anti-aggregatory action of PGI_2 on platelets is related to its ability to elevate the intracellular cAMP content. cAMP levels in vascular smooth muscle and endothelium also rise in response to PGI_2 [56]. Remarkably, reduced cAMP levels are observed in ischemic cardiac tissue after treatment with PGI_2 [50, 57]. This could indicate that interaction of PGI_2 with receptors on the sarcolemma of myocytes leads to an opposite response. Alternatively, PGI_2 also reduces ischemia-induced catecholamine release [58] and, hence, might inhibit the catecholamine-induced activation of adenylate cyclase and subsequent rise in cAMP.

Several other mechanisms have been proposed to explain the observed effects of PGI_2 on myocytes. Beside reducing the cAMP level PGI_2 possesses membrane stabilizing properties, which might promote lysosomal stability [58, 59]. Adverse cellular effects include the inhibition of the sarcolemmal Na^+/K^+-ATPase and facilitation of Ca^{2+} transport across cellular membranes [55, 60, 61]. At present it is unclear which effects will prevail at (patho)physiological concentrations of PGI_2.

Acknowledgements

Supported by a grant from Medigon/NWO (nr. 900-516-091).

References

1. Schwartzmann M, Carroll MA, Ibraham NG, Ferreri NR, Songu-Mize E, McGiff JC: Renal arachidonic acid metabolism. The third pathway. Hypertension 7 (suppl 1): 136–144, 1985
2. Needleman P, Turk J, Jakschik BA, Morrison AR, Lefkowith JB: Arachidonic acid metabolism. Ann Rev Biochem 55: 69–102, 1986
3. Hsueh W, Needleman P: Sites of lipase activation and prostaglandin synthesis in isolated, perfused hearts and hydronephrotic kidneys. Prostaglandins 16: 661–681, 1978
4. Karmazyn M, Moffat MP: Calcium ionophore stimulated release of leukotriene C_4-like immunoreactive material from cardiac tissue. J Mol Cell Cardiol 16: 1071–1073, 1984
5. Engels W, Van Bilsen M, Van der Vusse GJ, Willemsen PHM, Coumans WA, Kamps MAF, Endert J, Reneman RS: Influence of intracellular Ca^{2+}-overload on eicosanoid synthesis of the myocardium. Basic Res Cardiol 82 (suppl 1): 245–252, 1987
6. Coker SJ, Ledingham MA, Parratt JR, Zeitlin IJ: Thromboxane B2 and 6-keto-$PGF_{1\alpha}$ release into cornary venous blood: effect of coronary artery occlusion. J Physiol 316: 12p, 1981
7. De Deckere EAM, Nugteren DH, Ten Hoor F: Prostacyclin is the major prostaglandin released from the isolated rabbit and rat heart. Nature 268: 160–163, 1977
8. Karmazyn M: Reduction of enzyme release from reperfused ischemic hearts by steroidal and non-steroidal prostaglandin synthesis inhibitors. Prost Leuk Med 11: 299–315, 1983
9. Van Bilsen M, Engels W, Willemsen PHM, Coumans WA, Van der Vusse GJ, Reneman RS: Arachidonic acid accumulation and eicosanoid synthesis during ischemia and reperfusion of isolated rat hearts. Prog Appl Microcirc 12: 236–243, 1987
10. De Deckere EAM: Effects of prostaglandins on coronary flow rate and left ventricular work in isolated rat heart. Eur J Pharmacol 58: 211–213, 1979
11. Edlund A, Berglund B, Van Dorne D, Kaijser L, Nowak J, Patrono C, Sollevi A, Wennmalm A: Coronary flow regulation in patients with ischemic heart disease: release of purines and prostacyclin and the effect of inhibitors of prostaglandin formation. Circulation 71: 1113–1120, 1985

12. Prosdomici M, Finesso M, Gorio A, Languino LR, Del Maschio A, Castagnoli MN, De Gaetano G, Dejana E: Coronary and systemic 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 during myocardial ischemia in dog. Am J Physiol 248: H493–H499, 1985
13. Reneman RS, Prinzen FW, Engels W, Van Bilsen M, Van der Vusse GJ: Arachidonic acid metabolism during myocardial ischemia. Prog Appl Microcirc 12: 152–169, 1987
14. Berger HJ, Zaret BL, Speroff L, Cohen LS, Wolfson S: Regional cardiac prostaglandin release during myocardial ischemia in anesthetized dogs. Circ Res 38: 566–571, 1976
15. Coker SJ, Marshall RJ, Parratt JR, Zeitlin IJ: Does the local myocardial release of prostaglandin E_2 or $F_{2\alpha}$ contribute to the early consequences of acute myocardial ischaemia. J Mol Cell Cardiol 13: 425–434, 1981
16. Sakai K, Ito T, Ogawa K: Roles of endogenous prostacyclin and thromboxane A_2 in the ischemic canine heart. J Cardiovasc Pharmacol 4: 129–135, 1982
17. Tanabe M, Terashita Z, Fujiwara S, Shimamoto N, Goto N, Nishikawa K, Hirata M: Coronary circulatory failure and thromboxane A_2 release during coronary occlusion and reperfusion in anaesthetised dogs. Cardiovasc Res 16: 99–106, 1982
18. Otani H, Engelman RM, Rousou JA, Breyer RH, Das DK: Enhanced prostaglandin synthesis due to phospholipid breakdown in ischemic-reperfused myocardium. J Mol Cell Cardiol 18: 953–961, 1986
19. Bolton HS, Chanderbhan R, Bryant RW, Bailey JM, Weglicki WB, Vahouny GV: Prostaglandin synthesis by adult heart myocytes. J Mol Cell Cardiol 12: 1287–1298, 1980
20. Hohl CM, Roesen P: The role of arachidonic acid in rat heart cell metabolism. Biochim Biophys Acta 921: 356–363, 1987
21. Gerritsen ME, Cheli CD: Arachidonic acid and prostaglandin endoperoxide metabolism in isolated rabbit coronary microvessels and isolated and cultivated coronary microvessel endothelial cells. J Clin Invest 72: 1658–1671, 1983
22. Jackson RM, Chandler DB, Fulmer JD: Production of arachidonic acid metabolites by endothelial cells in hyperoxia. J Appl Physiol 61: 584–591, 1986
23. Revtyak GE, Johnson AR, Campbell WB: Cultured bovine coronary arterial endothelial cells synthesize HETEs and prostacyclin. Am J Physiol 254: C8–C19, 1988
24. Cole OF, Fan TPD, Lewis GP: Release of eicosanoids from cultured rat aortic endothelial cells; studies with arachidonic acid and calcium ionophore. Cell Biol Int Rep 10: 407–413, 1986
25. Brandt R, Nowak J, Sonnenfeld T: Prostaglandin formation from exogenous precursor in homogenates of human cardiac tissue. Basic Res Cardiol 79: 135–141, 1984
26. Lands WEM: The biosynthesis and metabolism of prostaglandins. Ann Rev Physiol 41: 633–652, 1979
27. Van der Vusse GJ, Roemen THM, Flameng W, Reneman RS: Serum-myocardium gradients of non-esterified fatty acids in dog, rat and man. Biochim Biophys Acta 752: 361–370, 1983
28. Van der Vusse GJ, Prinzen FW, Van Bilsen M, Engels W, Reneman RS: Accumulation of lipids and lipid-intermediates in the heart during ischemia. Basic Res Cardiol 82 (suppl 1): 157–168, 1987
29. Van Bilsen M, Van der Vusse GJ, Willemsen PHM, Coumans WA, Roemen THM, Reneman RS: Lipid alterations in isolated, working rat hearts during ischemia and reperfusion: its relation to myocardial damage. Circ Res 64: 304–314, 1989
30. Gerritsen ME: Eicosanoid production by the coronary microvascular endothelium. Fed Proc 46: 47–53, 1987
31. Salmon JA: Inhibition of arachidonic acid metabolism. In: Cardiovascular pharmacology of the prostaglandins. AG Herman, PM Vanhoutte, H Denolin, A Goossens (eds). Raven press, New York, 1982, pp 7–22
32. Karmazyn M: Contribution of prostaglandins to reperfusion-induced ventricular failure in isolated rat hearts. Am J Physiol 251: H133–H140, 1986
33. Chien KR, Han S, Sen A, Buja LM, Willerson JT: Accumulation of unesterified arachidonic acid in ischemic canine myocardium. Circ Res 54: 313–322, 1984
34. Hsueh W, Isakson PC, Needleman P: Hormone selective lipase activation in the isolated rabbit heart. Prostaglandins 13: 1073–1091, 1977
35. Paul J, Kinsella JE: Effects of bradykinin and bovine serum albumin on arachidonic acid and prostaglandin release from perfused rat heart. Prost Leuk Med 11: 419–430, 1983
36. Lefer AM: Leukotrienes as mediators of ischemia and shock. Biochem Pharmacol 35: 123–127, 1986
37. Schroer K: Eicosanoids and myocardial ischemia. Basic Res Cardiol 82 (suppl 1): 235–243, 1987
38. Schroer K: Prostaglandine und verwandte Verbindungen: Bildung, Funktion und pharmakologische Beeinflussung. Georg Thieme Verlag Stuttgart, New York, 1984
39. Mullane K, Barst S, McGiff JC: Leukotrienes in myocardial ischemia. In: Leukotrienes in cardiovascular and pulmonary function. AM Lefer, MH Gee (eds) Alan R Liss Inc., New York 1985, pp 127–140
40. Bednar M, Smith B, Pinto A, Mullane KM: Nafazatrom-induced salvage of ischemic myocardium in anesthetized dogs is mediated through inhibition of neutrophil function. Circ Res 57: 131–141, 1985
41. Toki Y, Hieda N, Torii T, Hashimoto H, Ito T, Ogawa K, Satake T: The effects of lipoxygenase inhibitor and peptido-leukotriene antagonist on myocardial injury in a canine coronary occlusion-reperfusion model. Prostaglandins 35: 555–571, 1988
42. Ikeda U, Toyo-oka T, Arisaka H, Hosoda S: Stimulated synthesis of prostaglandin E_2 or leukotriene C_4 from myocardial cells is not a cause but a result of their injury under hypoxia. J Mol Cell Cardiol 19: 523–526, 1987
43. Hock CE, Brezinski ME, Lefer AM: Anti-ischemic actions of a new tromboxane receptor antagonist SQ-29,548, in acute myocardial ischemia. Eur J Pharmacol 122: 213–219, 1986
44. Schroer K, Thiemermann C: Treatment of acute myocar-

dial ischaemia with a selective antagonist of thromboxane receptors (BM 13.177). Br J Pharmacol 87: 631–637, 1986
45. Pieper GM, Farber NE, Gross GJ: Metabolic protection of the reperfused canine endocardium by the thromboxane synthetase inhibitor, dazoxiben. Prost Leuk Ess Fatty Acids 33: 13–22, 1988
46. Karmazyn M, Dhalla NS: Physiological and pathophysiological aspects of cardiac prostaglandins. Can J Physiol Pharmacol 61: 1207–1225, 1983
47. Ogletree ML, Lefer AM, Smith JB, Nicolaou KC: Studies on the protective effect of prostacyclin in acute myocardial ischemia. Eur J Pharmacol 56: 95–103, 1979
48. Ribeiro LGT, Brandon TA, Hopkins DG, Reduto LA, Taylor AA, Miller RR: Prostacyclin in experimental myocardial ischemia: Effects on hemodynamics, regional myocardial blood flow, infarct size and mortality. Am J Cardiol 47: 835–840, 1981
49. Jugdutt BI, Hutchins GM, Bulkley BH, Becker LC: Dissimilar effects of prostacyclin, prostaglandin E_1, and prostaglandin E_2 on myocardial infarct size after coronary occlusion in conscious dogs. Circ Res 49: 685–700, 1981
50. Pissarek M, Goos H, Noehring J, Graff J, Buller G, Beyerdoerfer I, Mest HJ, Lindau KF, Krause EG: Prostacyclin and iloprost: equal efficiency in preserving high energy phosphates in the dog heart following coronary artery ligation. Basic Res Cardiol 82: 566–575, 1987
51. Farber NE, Pieper GM, Thomas JP, Gross GJ: Beneficial effects of iloprost in the stunned canine myocardium. Circ Res 62: 204–215, 1988
52. Araki H, Lefer AM: Role of prostacyclin in the preservation of ischemic myocardial tissue in the perfused cat heart. Circ Res 47: 757–763, 1980
53. Nayler WG, Purchase M, Dusting GJ: Effect of prostacyclin infusion during low-flow ischaemia in the isolated perfused rat heart. Basic Res Cardiol 79: 125–134, 1984
54. Edlund A, Sahlin K, Wennmalm A: Effect of prostacyclin on the severity of ischaemic injury in rabbit hearts subjected to coronary ligation. J Mol Cell Cardiol 18: 1067–1076, 1986
55. Moffat MP: Concentration-dependent effects of prostacyclin on the response of the isolated guinea pig heart to ischemia and reperfusion: possible involvement of the slow inward current. J Pharmacol Exp Ther 242: 292–299, 1987
56. Vane JR, Gryglewski RJ, Botting RM: The endothelial cell as a metabolic and endocrine organ. Trends Pharmacol Sci 8: 491–496, 1987
57. Roesen R, Roesen P, Ohlendorf R, Schroer K: Prostacyclin prevents ischemia-induced increase of lactate and cAMP in ischemic myocardium. Eur J Pharmacol 69: 489–491, 1981
58. Schroer K, Darius H, Addicks K, Koester R, Smith EF: PGI_2 prevents ischemia-induced alterations in cardiac catecholamines without influencing nerve-stimulation-induced catecholamine release in non-ischemic conditions. J Cardiovasc Pharmacol 4: 741–748, 1982
59. Okamatsu S, Lefer AM: Cardiac effects of prostaglandins during global ischemia in isolated perfused cat hearts. Prost Leuk Med 8: 1–10, 1982
60. Karmazyn M, Tuana BS, Dhalla NS: Effect of prostaglandins on rat heart sarcolemmal ATPases. Can J Physiol Pharmacol 59: 1122–1127, 1981
61. Karmazyn M: Prostaglandins stimulate calcium-linked changes in heart mitochondrial respiration. Am J Physiol 251: H141–H147, 1986

Address for offprints: M. van Bilsen, Department of Physiology, University of Limburg, P.O. Box 616, 6200 MD Maastricht, The Netherlands