

## The role of PPAR in myocardial response to ischemia in normal and diseased heart

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**Abstract.** Peroxisome proliferator-activated receptors (PPAR), ligand-activated transcription factors, belong to the nuclear hormone receptor superfamily regulating expression of genes involved in different aspects of lipid metabolism, inflammation and cardiac energy production. Activation of PPAR- $\alpha$  isoform by its natural ligands, fatty acids (FA) and eicosanoids, promotes mitochondrial FA oxidation as the primary ATP-generating pathway. On the other hand, PPAR- $\gamma$  regulates lipid anabolism or storage, while, until recently, the function of PPAR- $\beta/\delta$  has been less explored. Under conditions associated with acute or chronic oxygen deprivation, PPAR- $\alpha$  modulates expression of genes that determine substrate switch (FA vs. glucose) aimed at maintenance of basic cardiac function. Although PPAR- $\alpha$  and PPAR- $\gamma$  synthetic agonists, hypolipidemic and antidiabetic drugs, have been reported to protect the heart against ischemia/reperfusion injury, it is still a matter of debate whether PPAR activation plays a beneficial or detrimental role in myocardial response to ischemia, in particular, in pathological conditions. This article reviews some findings demonstrating the impact of PPAR activation on cardiac resistance to ischemia in normal and pathologically altered heart. Specifically, it addresses the issue of susceptibility to ischemia in the diabetic myocardium, with particular regards to the role of PPAR. Finally, involvement of PPAR in the mechanisms of lipid-independent cardioprotective effects of some hypolipidemic drugs is also discussed.

**Key words:** Peroxisome proliferator-activated receptors — Myocardial ischemia — Cardioprotection — Hypolipidemic drugs — HMG-CoA reductase inhibitors — Pleiotropic effects

**Abbreviations:** CPT1, carnitine palmitoyltransferase; ERK, extracellular signal regulated kinase; FA, fatty acids; GLUT4, glucose transporter; I/R, ischemia/reperfusion; ISGF-RE, interferon stimulated gene factor response element; MAPK, mitogen-activated protein kinases; MCAD, medium-chain acyl-CoA dehydrogenase; NF- $\kappa$ B-RE, NF- $\kappa$ B response element; PDK4, pyruvate dehydrogenase; PPAR, peroxisome proliferator-activated receptors; PPRE, PPAR response element; RXR, retinoid X receptor; STAT, signal transducer and activator of transcription; TRE, TPA (12-O-tetradecanoyl-phorbol-13-acetate) response element.

### Introduction

Peroxisome proliferator-activated receptors (PPAR) are ligand-activated transcription factors that belong to the nu-

clear hormone receptor superfamily regulating expression of genes involved in different aspects of lipid metabolism (Desvergne and Wahli 1999; Kliewer et al. 2001), energy production (Barger and Kelly 2000) and inflammation (Michalik and Wahli 2006). All three isoforms of PPAR detected so far PPAR- $\alpha$ , PPAR- $\beta/\delta$  and PPAR- $\gamma$ , encoded by separate genes, are expressed in many species including rodents (Kliewer et al. 1994) and humans (Greene et al. 1995). They differ in their tissue distribution, ligand specificity and cofactor interac-

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tions (Braissant et al. 1996; Escher et al. 2001). Therefore, PPAR isoforms regulate different sets of genes and there are different biological consequences of their stimulation. PPAR- $\alpha$  has been recognized as the central regulator of mitochondrial fatty acids (FA) catabolism, whereas PPAR- $\gamma$  is believed to regulate lipid anabolism or storage. Until recently, the function of PPAR- $\beta/\delta$  was relatively less explored. However, several lines of evidence suggest that all three isoforms modulate cardiac energy metabolism (Desvergne and Wahli 1999; Kliewer et al. 2001). Nevertheless, it is still a matter of debate whether PPAR activation plays a beneficial or detrimental role in the setting of ischemia/reperfusion (I/R), in particular in pathologically altered myocardium. Conflicting findings have documented both, negative impact of PPAR- $\alpha$  up-regulation on myocardial functional recovery upon I/R (Panagia et al. 2005; Sambandam et al. 2006), in particular during early reperfusion (Kantor et al. 2000), and beneficial effects of PPAR- $\alpha$  and PPAR- $\gamma$  agonists on I/R damage (Taberner et al. 2002; Wayman et al. 2002; Yue et al. 2003; Yeh et al. 2006). This contradiction is apparently related to the fact that PPAR activation may improve myocardial function *via* metabolic or other, metabolic-independent, actions.

### Tissue distribution and function of PPAR

Main tissue distribution and physiological effects of PPAR isoforms are illustrated in Table 1. Two of the three PPAR isoforms, PPAR- $\alpha$  and PPAR- $\beta/\delta$  are abundantly expressed in tissues with high level of FA oxidation (FAO) including heart, liver, kidney, skeletal muscle and pancreas (Braissant et al. 1996; Gilde et al. 2003). PPAR- $\gamma$  (and its splice variants) is mainly associated with adipose tissue and macrophages, with a low level of more ubiquitous expression in liver, heart, skeletal muscle and bone marrow (Escher et al. 2001). PPAR- $\beta/\delta$  is abundantly and ubiquitously expressed at much higher levels than PPAR- $\gamma$  and PPAR- $\alpha$  (Kliewer et al. 1994). It is important to note that tissue expression of all three PPAR isotypes may vary under different physiological and/or pathological conditions.

Heart tissue normally utilizes FA as the major energy source, and PPAR- $\alpha$  regulates genes encoding enzymes of FA transport/uptake and utilization *via*  $\beta$ -oxidation in mitochondria (Finck 2007). Activation of PPAR- $\alpha$  by its natural ligands (long-chain FA, eicosanoids) promotes mitochondrial FAO as the primary ATP-generating pathway in the normal adult myocardium (Barger and Kelly 2000; Finck 2007). Moreover, under physiological and pathological conditions associated with acute or chronic oxygen deprivation, PPAR- $\alpha$  modulates expression of genes that determine myocardial substrate selection (FA *versus* carbohydrates) in order to maintain adequate production of energy and preserve basic cardiac function (Huss and Kelly 2004). In ad-

dition, involvement of PPAR in anti-inflammatory response in different tissues has been also recognized (Delerive et al. 2001; Smeets et al. 2007).

### Mechanisms of action of PPAR

#### *Natural and synthetic PPAR ligands*

Upon binding to PPAR, different ligands can induce stimulatory or inhibitory responses depending on the nature of the specific target gene and its cellular location. Both natural and synthetic compounds have been recognized as PPAR ligands. Although many FA are capable of activating all three PPAR isoforms, some preference for specific FA by each PPAR has been demonstrated (reviewed by Collino et al. 2008). The long-chain polyunsaturated FA and their oxidized derivatives, especially eicosanoids such as 8-S-hydroxyeicosatetraenoic acid (8-S-HETE), leukotriene B<sub>4</sub> (LTB<sub>4</sub>), and arachidonate monooxygenase metabolite epoxyeicosatrienoic acids have been shown to potently activate PPAR- $\alpha$  with high affinity (Feige et al. 2006). PPAR- $\gamma$  can be activated by several prostanoids, such as 15-deoxy-12,14-prostaglandin J<sub>2</sub> (15d-PGJ<sub>2</sub>) and 12- and 15-hydroxy-eicosatetraenoic acid (12- and 15-HETE), which are derivatives of arachidonic acid synthesized through the lipoxygenase pathway (Theocharis et al. 2004). Prostaglandin 15d-PGJ<sub>2</sub> is not only the most potent natural ligand for PPAR- $\gamma$  identified to date, but also by far the most commonly used naturally occurring PPAR- $\gamma$  agonist (Forman et al. 1997). In addition to PPAR- $\gamma$  naturally occurring agonists produced by human body, flavonoids psibaptigenin and hesperidin found in plants were identified as strong PPAR- $\gamma$  agonists (Salam et al. 2008).

Several synthetic agonists of PPAR- $\alpha$  and PPAR- $\gamma$  are known as marketed drugs used in the treatment of hypertriglyceridemia and diabetes mellitus, respectively (Baltantyne et al. 2003; Tenenbaum et al. 2004). Hypolipidemic drugs fibrates (e.g. fenofibrate, clofibrate) are well-known synthetic ligands for PPAR- $\alpha$  (Theocharis et al. 2004). Fibrates activate PPAR- $\alpha$  leading to increased expression of lipid metabolizing enzymes that effectively lower serum lipid levels, in particular triacylglycerides, in humans.

The most widely used synthetic PPAR- $\gamma$  agonists belong to the thiazolidinedione (TZD) or glitazone class of anti-diabetic drugs used in the treatment of type-2 diabetes. The two available TZDs, rosiglitazone and pioglitazone, are currently used alone or in combination with other oral anti-diabetic agents (Theocharis et al. 2004). These drugs are known as insulin sensitizers stimulating the tissue uptake of glucose in the diabetics (Sidell et al. 2002), however, their action extends far beyond their hypoglycemic activity (Khandoudi et al. 2002; Shiomi et al. 2002; Lee et al. 2003). Table 2 summarizes currently known PPAR modulators, as

**Table 1.** Tissue distribution and physiological effects of PPAR isoforms

PPAR isoform	Tissue distribution	Effects
PPAR- $\alpha$	Liver, skeletal muscle, heart, kidney, adipose tissue	FA metabolism, cell cycle, control of inflammation and apoptosis
PPAR- $\beta/\delta$	Ubiquitously	FA metabolism, wound healing, control of inflammation
PPAR- $\gamma$ 1	All tissues including heart, muscle, kidney	Adipogenesis, lipid storage, control of inflammation and apoptosis, increased insulin sensitivity and glucose disposal
PPAR- $\gamma$ 2	Adipose tissue	
PPAR- $\gamma$ 3	Macrophages	

well as clinically important PPAR- $\alpha$  and PPAR- $\gamma$  agonists, fibrates and glitazones, respectively.

#### *Transcriptional transactivation*

Upon activation by endogenous or synthetic ligands, PPAR form obligate heterodimers with the 9-cis retinoic acid receptors (retinoid X receptor, RXR). The resulting complex undergoes a conformational change which allows binding of the heterodimer to a DNA sequence in the promoter region of target genes known as the PPAR response element (PPRE) followed by the induction of gene transcription (Kliwer et al. 1992; Forman et al. 1997) and synthesis of the respective gene products. When both PPAR and RXR are activated simultaneously, it results in significant synergistic enhancement of gene transcription (Kliwer et al. 1992). The search for PPAR target genes with identified PPREs has led to the identification of numerous genes involved in lipid metabolism, oxidative stress and the inflammatory response (Desvergne and Wahli 1999; Delerive et al. 2001; Tan et al. 2005; Finck 2007), as well as genes responsible for insulin signaling and glucose metabolism (Grossman and Lessem 1997; Oshida et al. 1999).

#### *Transcriptional transrepression*

In addition to PPAR transactivation, stimulation of PPAR can also negatively regulate gene expression in a ligand-dependent manner by inhibiting the activities of other transcription factors, such as activated protein-1 (AP-1), nuclear factor-kappaB (NF- $\kappa$ B), nuclear factor of activated T-cells (NFAT) or signal transducer and activator of transcription (STAT) *via* mechanism known as ligand-dependent transrepression (Abdelrahman et al. 2005). In contrast to transcriptional activation, transrepression does not involve binding of PPAR to response elements of the target genes but direct interaction with other transcription factors and co-repressors or modulation of kinase activity.

Research suggests that PPAR may exert beneficial effects by negatively regulating the expression of pro-inflammatory genes in inflammation-related diseases including myocardial ischemia/reperfusion injury (Abdelrahman et al. 2005). Several mechanisms have been suggested to

account for this activity including ligand-independent repression of the transcription of target genes *via* binding of PPAR to response element in the absence of ligands and recruitment of the co-repressor complexes (reviewed by Collino et al. 2008).

#### *Regulation of PPAR activity*

Many proteins act as co-activators or co-repressors that regulate the ability of PPAR to either stimulate or repress gene transcription. In the unbound state, PPAR/RXR heterodimers are associated with co-repressors, which prevent gene transcription. However, once a ligand binds to the receptor, a conformational change occurs that not only facilitates co-repressor dissociation, but also the recruitment of several positive co-activators that initiates a sequence of events ultimately leading to gene transcription (Shibata et al. 1997).

Although co-activators and co-repressors appear to be the major factors responsible for regulation of PPAR activity, these receptors can also be modulated by mitogen-activated protein kinase (MAPK)-induced phosphorylation. In fact, phosphorylation by extracellular signal regulated kinases (ERK1/2) has been found to repress PPAR- $\alpha$  activity (Barger and Kelly 2000; Barger et al. 2000), while phosphorylation induced by p38-MAPK enhances PPAR- $\alpha$ -mediated gene expression (Barger et al. 2001).

Figure 1 summarizes regulation of PPAR function in the cell, control of gene expression and PPAR-mediated effects.

### **PPAR function and the outcome of myocardial ischemia/reperfusion injury**

Delivery of oxygen and metabolic substrates *via* coronary circulation is essential for normal cardiac function, and its cessation leads within minutes to irreversible cellular injury. The duration of ischemia and the extent of metabolic and structural alterations in the myocardium are the main factors that determine the progress towards cell death (by mechanisms of necrosis or apoptosis) or cell survival. Restoration of blood flow in the previously occluded coronary arteries is undoubtedly the main prerequisite of the heart rescue. However,

**Table 2.** Naturally occurring and synthetic PPAR- $\alpha$  and PPAR- $\gamma$  modulators

	PPAR- $\alpha$ modulators	PPAR- $\gamma$ modulators
Naturally occurring agonists	Fatty acids Eicosanoids Leukotriene B4	Fatty acids Eicosanoids Prostaglandin Flavonoids: $\psi$ -baptigenin, hesperidin
Synthetic agonists	Fibrates: fenofibrate, clofibrate, bezafibrate, gemfibrozil WY14643, GW7647	Thiazolidinediones (glitazones): rosiglitazone, pioglitazone, ciglitazone
Synthetic antagonists	MK-886	GW9662

reperfusion may have injurious components and limit the recovery of the tissue through the induction of “reperfusion injury” (Braunwald and Kloner 1985). I/R injury represents a clinically relevant problem associated with restoration of blood supply that occurs during thrombolysis, percutaneous coronary intervention and coronary artery bypass graft surgery (Roberto and Prado 2002; Rodríguez-Sinovas et al. 2007). I/R injury is a complex cascade of events, where oxidative stress and inflammatory response play the pivotal role (Turer and Hill 2010) and besides other factors involve activation of NF- $\kappa$ B as one of the central processes (Hall et al. 2006), in particular in the *ex vivo* perfused heart (Li et al. 1999).

The role of PPARs in the pathogenesis of a variety of heart disorders including acute myocardial I/R is a matter of controversy and still remains unclear. Gene expression of PPAR- $\alpha$  declines in chronically hypoxic heart resulting in a substrate switch from FA to glucose that has been considered as an adaptive response (Barger et al. 2000; Razeghi et al. 2001). In line, experimental overexpression of PPAR- $\alpha$  was found to be related to the impaired cardiac recovery after ischemia (Sambandam et al. 2006). It appears that in long-term processes, such as myocardial hypoxia and/or hypertrophy linked with limitations in oxygen supply, glucose as a fuel may be beneficial for the heart by decreasing oxygen consumption (Barger and Kelly 2000). Moreover, chronic activation of PPAR- $\alpha$  (and increased rates of FAO at the expense of glucose oxidation) may be detrimental to the heart during postischemic reperfusion possibly due to FAO-induced oxidative stress (Sambandam et al. 2006).

On the other hand, other studies indicated that targeted deletion of PPAR- $\alpha$  resulted in increased serum levels of free FA and a larger size of infarction in mice subjected to ischemic challenge (Yue et al. 2003). In acute settings of I/R, decrease of PPAR- $\alpha$  and corresponding metabolic effects were observed in a rat *ex vivo* model of 30-min ischemia/2-h reperfusion (Tian et al. 2006) and in the *in vivo* mice. In these models, reversal of down-regulation of PPAR- $\alpha$  and its target genes responsible for the metabolic fuel shifts (decreased FAO and increased glucose oxidation) improved postischemic myocardial contractile recovery and reduced

the size of infarction (Yue et al. 2003). In line, in our study in the isolated rat heart, 30-min global ischemia significantly decreased mRNA and protein levels of PPAR- $\alpha$  and their further decline observed following 2-h reperfusion was accompanied by the development of irreversible myocardial injury (Ravingerová et al. 2009).

There is no clear consensus on whether attenuation of I/R-induced down-regulation of PPAR- $\alpha$  and FAO is beneficial or detrimental to the heart. The discrepancy in the results may arise from the different substrate availability in the different experimental models (ischemia/reperfusion, *in vivo* versus *in vitro* protocols). Although FAO is an important source of energy production during the basic conditions, glucose uptake may be crucial during ischemia. It is believed that partial inhibition of FAO and a substrate switch from FA to glucose (Barger and Kelly 2000) improves functional recovery of the heart upon reperfusion (Fragasso et al. 2003) while overexpression of PPAR- $\alpha$  impairs postischemic cardiac recovery (Sambandam et al. 2006). Thus, pharmacological interventions that increase glucose oxidation and suppress FAO appear to be beneficial for the recovery of the myocardium previously subjected to I/R (Kantor et al. 2000; Panagia et al. 2005). In the long-term, however, this switch may become detrimental as less ATP is generated *per* mole of glucose oxidized, and lipid accumulation and lipotoxicity of the myocardium may develop (Barger and Kelly 2000). The controversy regarding the role of PPAR- $\alpha$  in the heart suggests that the function of this transcription factor might not be the same in different cardiac pathologies or in their different stages and that the effects other than lipid metabolism might be also involved. Figure 2 shows potential involvement of PPARs in the pathophysiological mechanisms of ischemia/reperfusion injury.

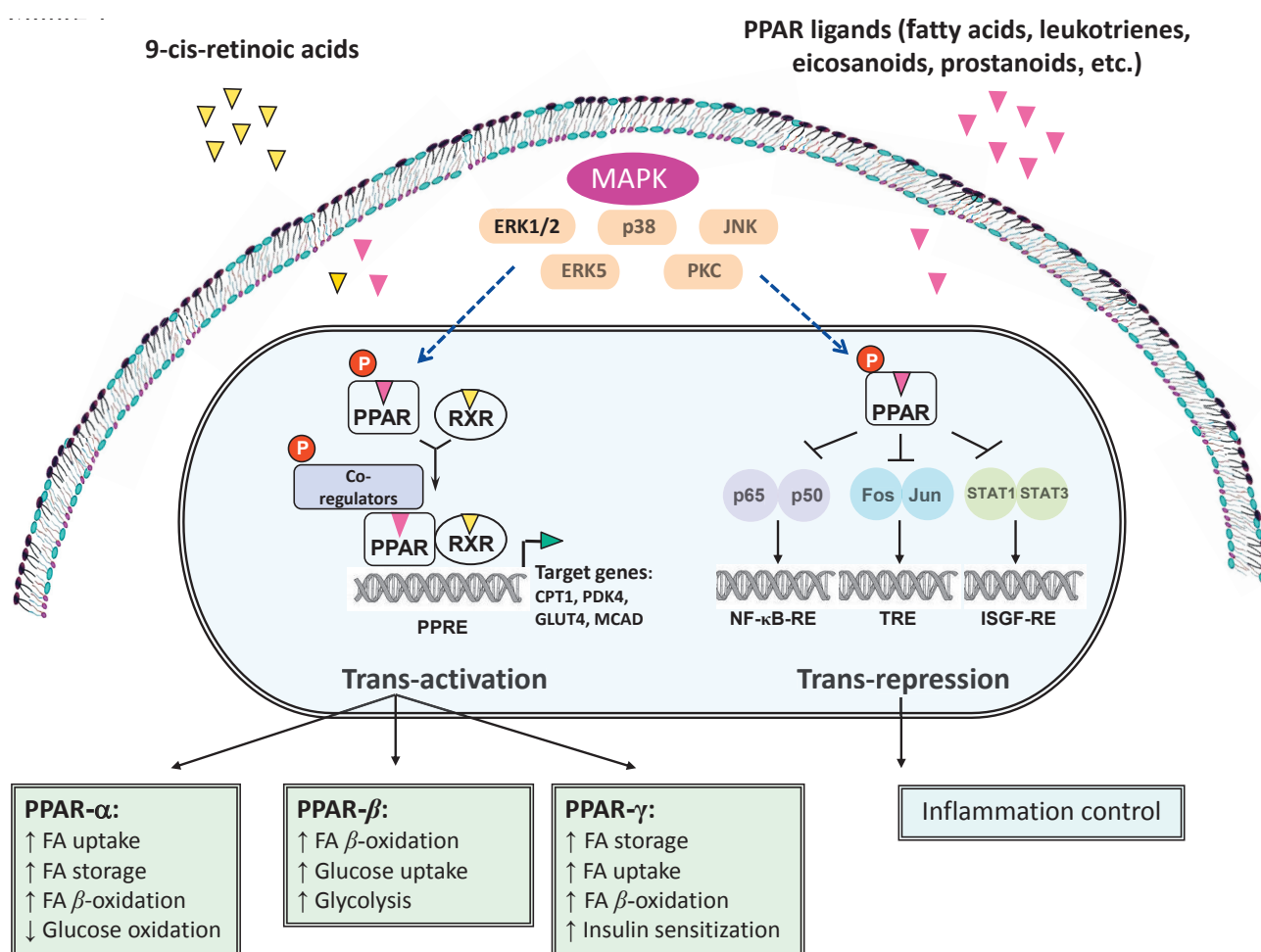
#### PPAR and endogenous protection against ischemia/reperfusion

The role of PPAR in the mechanisms of endogenous protection against I/R injury is less documented, although

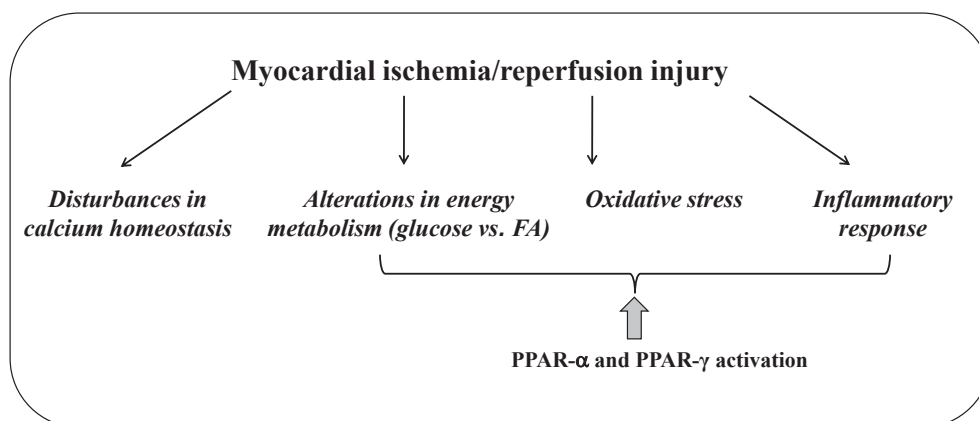
Takeda et al. (2001) demonstrated that PPAR- $\gamma$  agonists activated ERK1/2 pathway of MAP-kinases in vascular smooth muscle cells through phosphatidylinositol 3-kinase (PI3K). Cascades of ERK1/2 and PI3K and its effector protein kinase B (Akt) are implicated in protective mechanisms of ischemic preconditioning and other forms of intrinsic cardioprotection (Hausenloy et al. 2005; Ravingerová et al. 2007). It has also been hypothesized that PPAR activation prior to I/R could confer preconditioning-like protection to the myocardium (Wynne et al. 2005). Indeed, increased PPAR- $\gamma$  activity resulted in significant anti-infarct protection comparable with the effect of classical ischemic

preconditioning that appeared to involve both survival cascades (ERK1/2 and PI3K/Akt).

Moreover, it has been shown that PPAR- $\gamma$  participates in a delayed effect of preconditioning with endotoxin (lipopolysaccharide, LPS) on myocardial and renal I/R injury in rats (Collino et al. 2005; Sivarajah et al. 2005). In addition, pretreatment of rabbits with anaesthetic desflurane has been reported to induce overproduction of endogenous PPAR- $\gamma$  agonists, such as 15d-PGJ2 and others, resulting in a delayed infarct size-limiting protection (Lotz et al. 2011a). Recently, the involvement of both, PPAR- $\alpha$  and PPAR- $\gamma$  isoforms in the mechanisms of “remote” (renal ischemia-induced)



**Figure 1.** Regulatory pathways of PPARs in the cell and control of gene expression by PPARs. PPARs regulate gene expression by binding with RXR to specific DNA sequence elements (PPRE) located in the promoter region of target genes, thus regulating their transcription and synthesis of the respective gene products (Trans-activation). PPARs repress gene transcription (Trans-repression) by negatively interfering with transcription factors such as NF- $\kappa$ B, STATs, cJun and c-Fos in a DNA-binding independent manner. The activity of PPARs is also regulated by phosphorylation events through MAPK signaling. CPT1, carnitine palmitoyltransferase; ERK, extracellular signal regulated kinases; FA, fatty acids; GLUT4, glucose transporter; ISGF-RE, interferon stimulated gene factor response element; MAPK, mitogen-activated protein kinases; MCAD, medium-chain acyl-CoA dehydrogenase; NF- $\kappa$ B-RE, nuclear factor- $\kappa$ B response element; PDK4, pyruvate dehydrogenase; PPAR, peroxisome proliferator-activated receptors; PPRE, PPAR response element; RXR, retinoid X receptor; STATs, signal transducer and activator of transcription; TRE, TPA (12-O-tetradecanoylphorbol-13-acetate) response element.



**Figure 2.** Involvement of PPARs in pathophysiological mechanisms of ischemia/reperfusion injury. FA, fatty acids; PPAR, peroxisome proliferator-activated receptors.

preconditioning against myocardial infarction in rabbits *in vivo* coupled with an increased transcriptional activity of inducible NO synthase has also been documented (Lotz et al. 2011b).

#### Cardioprotective effects of exogenous PPAR agonists

Activation of PPAR- $\alpha$  with synthetic ligands has been shown to be cardioprotective in a setting of I/R as manifested by a reduced infarct size and improved postischemic recovery of contractile function in different *in vivo* and *ex vivo* models of I/R (Wayman et al. 2002; Yue et al. 2003; Tian et al. 2006). In this context, treatment with PPAR- $\alpha$  selective and potent agonist GW7647, that reversed I/R-induced down-regulation of PPAR- $\alpha$  and its target genes, attenuated myocardial contractile dysfunction and reduced the size of infarction (Yue et al. 2003). Similar cardioprotective effects, in conjunction with the metabolic effects, were observed in a rat *ex vivo* model of 30-min ischemia/2-h reperfusion after treatment with PPAR- $\alpha$  agonist clofibrate (Tian et al. 2006). These studies do not support the view of the beneficial role of FAO inhibition in the mechanisms of protection against acute I/R, at least in this experimental setting.

PPAR- $\alpha$  agonists fibrates have shown protection against myocardial I/R injury beyond their lipid-lowering properties (Wayman et al. 2002). Other potent hypolipidemic drugs, statins, are being also intensively studied in this respect. By inhibition of the enzyme HMG-CoA reductase statins have been reported to prevent the synthesis of isoprenoid intermediates of cholesterol biosynthesis pathway involved in posttranslational modification of small GTP-binding proteins, such as Ras, Rho, and Rac, which modulate a variety of cellular processes (Takemoto and Liao 2001), e.g., oxidative stress and inflammation (Van Linthout et al. 2007; Zhou et al.

2008; Adameová et al. 2009b), vascular endothelial dysfunction (Takemoto and Liao 2001) and the outcome of myocardial response to I/R (Adameová et al. 2006, 2009a). It is hypothesized that in the myocardium, treatment with statins induces preconditioning-like effects attributed to up-regulation of "survival" pathways, such as PI3K/Akt, ERK1/2 and eNOS (Di Napoli et al. 2001; Efthymiou et al. 2005; Merla et al. 2007). In addition, in the hearts of normocholesterolemic rats exposed to I/R after 5-days treatment with simvastatin, a remarkable elevation in PPAR- $\alpha$  gene expression coupled with an enhanced protein expression (3.3-fold and 2-fold increase in mRNA and protein levels, respectively) was observed in the myocardium of these animals at baseline and after 30-min global ischemia and 2-h reperfusion. This was accompanied by a significant reduction of the infarct size, improved contractile recovery and attenuation of severe ventricular arrhythmias (Ravingerová et al. 2009).

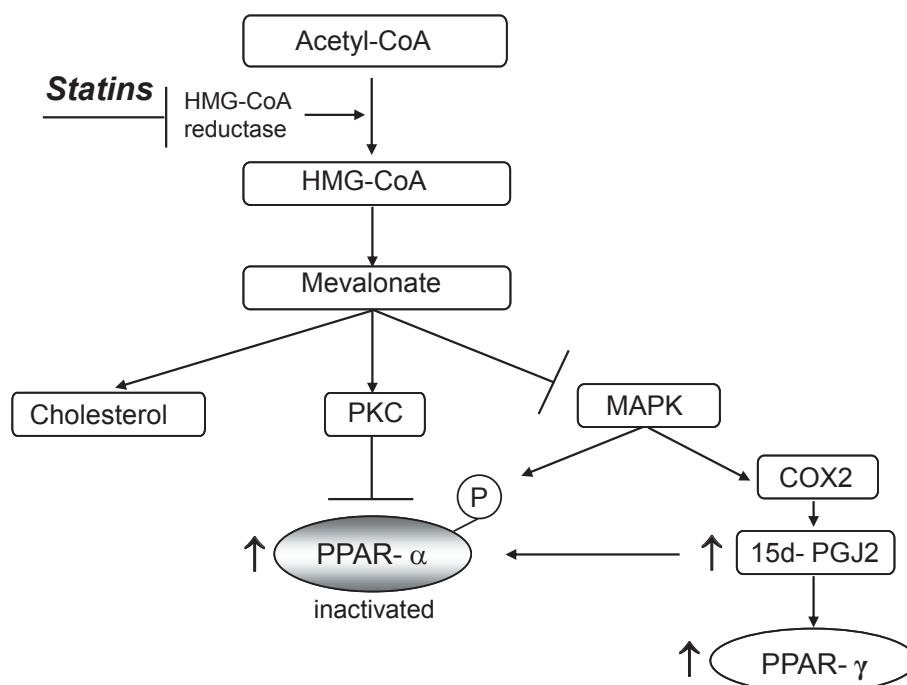
Although statins are not specific PPAR ligands, they have been reported to up-regulate PPAR- $\alpha$  in some cell types, such as human HepG2 hepatoma cells (Martin et al. 2001) or mice peritoneal macrophages (Paumelle et al. 2006) and to increase both PPAR- $\alpha$  expression and its protein levels in primary endothelial cells (Inoue 2000). Our findings provided evidence of the up-regulation of PPAR- $\alpha$  by statins in the myocardium, perhaps not *via* a direct agonistic mechanism. In support of the view that PPAR- $\alpha$  activation may underlie mechanisms of beneficial effects of statins against lethal myocardial injury in the hearts of normocholesterolemic animals, anti-infarct protection conferred by 5-days treatment with simvastatin in a rat *ex vivo* model (Adameová et al. 2009a; Ravingerová et al. 2009) was comparable with the effect of WY14643, a hypolipidemic compound that has been shown to protect rat myocardium against I/R injury (Bulhak et al. 2006) as one of the most potent and selective PPAR- $\alpha$  agonist (Forman et al. 1997). This is also in agree-

ment with the data documenting a beneficial effect of PPAR- $\alpha$  activation on cardiac I/R injury (Wayman et al. 2002; Yue et al. 2003; Tian et al. 2006) indicating that preserved FAO is important for the maintenance of adequate energy production under the conditions of restored coronary flow, when oxygen supply is no longer rate limiting.

#### PPAR and inflammation

Protective effects of PPAR agonists may be attributed not only to modulation of cardiac metabolism but also to inhibition of inflammation with the salutatory effects on the cardiac muscle (Diep et al. 2004; Smeets et al. 2007). In fact, in the experiments which have demonstrated beneficial effects of PPAR- $\alpha$  and - $\gamma$  agonists on the myocardial, cerebral and hepatal I/R injury, protection was attributed to the attenuation of oxidative stress and inflammatory response via inhibition of the activation of NF- $\kappa$ B (Delerive et al. 2000; Yue et al. 2003; Ogata et al. 2004; Collino et al. 2006; Yeh et al. 2006; Xu et al. 2008). Recent study by Collino et al. (2011) has demonstrated protective effects PPAR  $\beta/\delta$  agonist against myocardial I/R associated with suppression of proinflammatory cytokines and neutrophil accumulation. Lipophilic HMG-CoA reductase inhibitors exerted an anti-inflammatory effect *via* reduction of mRNA

levels for interleukin-1 $\beta$ , interleukin-6, cyclooxygenase-2, and p22phox by up-regulation of PPAR- $\alpha$  (and PPAR- $\gamma$ ) in primary endothelial cells (Inoue et al. 2000). Positive impact of statins on inflammatory processes may be mediated through the activation of both PPAR- $\alpha$  and PPAR- $\gamma$  (Inoue et al. 2002; Zelvyte et al. 2002). Research indicates that acute anti-inflammatory effect of simvastatin occurs through a mechanism involving inhibition of PKC- (and ERK1/2 cascade of MAPK)-induced phosphorylation (and inactivation) of PPAR- $\alpha$ , activation of PPAR- $\alpha$  (and PPAR- $\gamma$ ) *via* a cyclooxygenase (COX)-2-dependent increase in the levels of natural PPAR ligands 15d-PGJ<sub>2</sub>, as well as decreased transactivation of NF- $\kappa$ B (Inoue et al. 2002; Zelvyte et al. 2002; Paumelle et al. 2006; Yano et al. 2007). Figure 3 summarizes potential mechanisms of PPAR activation as a part of pleiotropic effects of statins induced by the inhibition of HMG-CoA reductase-mevalonate pathway. A remarkable similarity between the pleiotropic effects of statins (including anti-inflammatory and anti-oxidant effects) and the agonists of PPAR- $\alpha$  fibrates, suggests a mechanistic link between these two classes of drugs and similarity in their effects on PPAR- $\alpha$  (Tian et al. 2006; Paumelle and Staels 2008). Thus, an improved outcome of I/R injury in statin-treated normocholesterolemic animals may be also linked to anti-inflammatory effects of PPAR activation. In support



**Figure 3.** Schematic representation of the potential mechanisms of PPARs activation by statins through the inhibition of HMG-CoA reductase-mevalonate pathway of cholesterol biosynthesis. COX, cyclooxygenase; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; MAPK, mitogen-activated protein kinases; PGJ<sub>2</sub>, 15-deoxy-delta-12,14-prostaglandin J<sub>2</sub>; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptors.

of this, Planavila et al. (2005) have reported that atorvastatin treatment prevented both, the fall in the protein levels of PPAR- $\alpha$  and NF- $\kappa$ B activation in pressure overload-induced cardiac hypertrophy.

### **PPAR function in the diabetic heart: susceptibility to ischemia/reperfusion injury**

PPARs are up-regulated in the diabetic myocardium (Huss and Kelly 2004) that almost exclusively relies on FAO for energy production resulting in higher myocardial oxygen consumption. The latter, along with elevated circulating levels and uptake of FA, as well as excess myocardial lipid accumulation may predispose the heart to contractile dysfunction and failure.

Clinical and epidemiological studies clearly demonstrated that diabetic patients are at a higher risk of congestive heart failure and ischemic heart disease including myocardial infarction and rhythm disorders (Kannel 1985). It has been found that development of diabetes leads to oxidative stress (Singal et al. 2001; Maritim et al. 2003) and defects in the cell sarcolemmal and sarcoplasmic reticular membranes, as well as to alterations in the function of ion transport systems (Na<sup>+</sup>/K<sup>+</sup>- and Ca<sup>2+</sup>-ATPase, Na<sup>+</sup>/H<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> exchangers and Ca<sup>2+</sup> channels). The latter leads to abnormal Na<sup>+</sup> and Ca<sup>2+</sup> handling in the diabetic myocardium that might compromise its tolerance to ischemia (Pierce et al. 1990; Lee et al. 1992; Dhalla et al. 1998; Anzawa et al. 2006).

On the other hand, animal studies are not unequivocal and suggest that, besides higher myocardial vulnerability, diabetes mellitus may trigger adaptive processes leading to paradoxically enhanced ischemic tolerance. This is now considered as a form of "metabolic preconditioning" sharing some molecular pathways with endogenous cardioprotection in the non-diabetic heart. In particular, it has been demonstrated that susceptibility to I/R in the experimental model of streptozotocin (STZ)- or alloxan-induced diabetes mellitus is decreased similarly to the effect of preconditioning in the non-diseased animals documented by reduced size of infarction, improved contractile recovery, suppressed arrhythmogenesis and lower myocardial generation of reactive oxygen species during ischemia as compared with non-diabetic hearts (Ravingerová et al. 2003, 2010; Galagudza et al. 2007; Matejčková et al. 2008).

Potential mechanisms of preconditioning-like protection in the diabetic myocardium may involve along with antiapoptotic effects of high glucose itself acting as a preconditioning mimetic in the absence of insulin (Ricci et al. 2008), a higher activity of "survival" protein kinases ERK1/2 and PI3K/Akt in acutely diabetic myocardium (Strnisková et al. 2003; Xu et al. 2004; Tsang et al. 2005; Ma et al. 2006).

In addition, several other protective mechanisms, such as reduction in the levels of pro-inflammatory cytokines, increase in the cell survival factors (HIF1- $\alpha$ , VEGF) and angiogenesis, along with reduced fibrosis have been found to be activated in the acute phase of STZ-induced diabetes (Malfitano et al. 2010).

Although PPAR- $\gamma$  agonists, insulin-sensitizing drugs glitazones, are widely used for the control of glycemia in diabetic patients (Grossman and Lessem 1997), the role of PPAR in ischemia-induced myocardial injury in the diabetic myocardium still remains elusive (Nikolaidis and Levine 2004). Limited evidence suggests that increased resistance to ischemia in the experimental models of diabetes mellitus might be coupled with enhanced baseline and post I/R mRNA levels of PPARs in contrast to their marked down-regulation in non-diabetics (Ravingerová et al. 2009, 2010). The latter indicates that the maintenance of enhanced PPAR gene expression during I/R may contribute to improved outcome of myocardial I/R injury in the diabetic heart, at least in the early phase of the disease. Moreover, this protective effect of PPAR up-regulation might possibly involve not only metabolic effects of PPARs but also their anti-inflammatory and antioxidative effects (Delerive et al. 2001; Smeets et al. 2007), through the negative regulation of NF- $\kappa$ B (Abdelrahman et al. 2005), that might be of particular importance in the diabetic myocardium. In line, Khandoudi et al. (2002) have shown that cardioprotective effects of PPAR- $\gamma$  activation in diabetic rat hearts exposed to global I/R is associated with inhibition of Jun NH(2)-terminal kinase phosphorylation. Recently, Collino et al. (2011) have demonstrated that acute activation of PPAR- $\beta/\delta$  by its selective agonist GW0742 conferred protection against renal I/R injury in rats with STZ-induced diabetes. Protection of kidney by activated PPAR- $\beta/\delta$  in this model involved attenuation of neutrophil infiltration and decreased proinflammatory cytokine signaling (Collino et al. 2011).

### *Loss of ischemic tolerance in the diabetic heart*

It has been shown that hypercholesterolemia (HCH) abrogated cardioprotective effect of preconditioning in the non-diabetic heart *via* alterations in preconditioning-induced gene expression resulting in an enhanced oxidative/nitrosative stress signaling (Giricz et al. 2006; Kocsis et al. 2010). Similarly, this pathology appeared to be one of the reasons for the loss of enhanced ischemic tolerance in the diabetic rats on 7-day high fat-cholesterol diet (Adameová et al. 2007, 2009a). Thus, comorbidity, such as HCH, blunted infarct size-limiting effect in the Langendorff-perfused hearts and exacerbated severe ventricular arrhythmias in the open-chest *in vivo* diabetic-hypercholesterolemic animals. Moreover, HCH suppressed upregulation of myocardial PPAR gene expres-



sion in diabetics, in particular, it decreased mRNA levels of PPAR- $\gamma$  below those detected in non-diabetic controls both at baseline and after I/R (Ravingerová et al. 2010). In addition, in a similar model of STZ-induced diabetes, inhibition of PPAR- $\beta/\delta$  by its selective antagonist GSK0660 abrogated beneficial effects of PPAR- $\beta/\delta$  activation on renal I/R injury (Collino et al. 2011). These findings indicate that changes in PPAR gene expression might be involved in the adaptive protective mechanisms activated in the diabetic myocardium in the acute phase of the disease to counteract metabolic disorders, while loss of protection might be potentially related to concomitant HCH and down-regulation of PPAR promoting detrimental pro-inflammatory and oxidative effects.

### Summary

In conclusion, experimental data suggest that changes in gene expression of PPARs are involved in the pathophysiological mechanisms of myocardial injury and may modulate it in a distinct way dependent on the type and duration of cardiac pathology. Collectively, these data indicate that up-regulation of PPARs may underlie mechanisms of preconditioning-like effects observed in the normal animals subjected to different protocols of adaptation prior to sustained ischemia or induced by lipid-independent cardioprotective action of some hypolipidemic drugs in non-diseased myocardium. Likewise, enhanced PPAR activity might be implicated in the mechanisms of enhanced resistance to ischemia in the acute phase of experimental diabetes. Thus, PPARs might represent an important therapeutic target in the management of ischemic heart disease in patients with or without metabolic disorders. However, a more detailed elucidation of the role of PPARs in myocardial ischemic injury and cardioprotection requires further investigation.

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