The Use of Partial Fatty Acid Oxidation Inhibitors for Metabolic Therapy of Angina Pectoris and Heart Failure

Heinz Rupp[1], Angel Zarain-Herzberg[2], Bernhard Maisch[1]

Background: Partial fatty acid oxidation inhibitors have raised great interest since they are expected to counteract a dysregulated gene expression of hypertrophied cardiocytes. Some of these compounds have been developed for treating non-insulin-dependent diabetes mellitus and stable angina pectoris. A shift from fatty acid oxidation to glucose oxidation leads to a reduced gluconeogenesis and improved economy of cardiac work. An increased glucose oxidation can be achieved with the following enzyme inhibitors: etomoxir, oxfenicine, methyl palmoxirate, S-15176, metoprolol, amiodarone, perhexiline (carnitine palmitoyltransferase-1); aminocarnitine, perhexiline (carnitine palmitoyltransferase-2); hydrazonopropionic acid (carnitine-acylcarnitine translocase); MET-88 (gamma-butyrobetaine hydroxylase); 4-bromocrotonic acid, trimetazidine, possibly ranolazine (thiolases); hypoglycin (butyryl-CoA dehydrogenase); dichloroacetate (pyruvate dehydrogenase kinase).

Clinical Trials with trimetazidine and ranolazine showed that this shift in substrate oxidation has an antianginal action. Etomoxir and MET-88 improved the function of overloaded hearts by increasing the density of the Ca^{2+} pump of sarcoplasmic reticulum (SERCA2). The promoters of SERCA2 and alpha-myosin heavy-chain exhibit sequences which are expected to respond to transcription factors responsive to glucose metabolites and/or peroxisome proliferator-responsive element (PPAR) agonists. Further progress in elucidating novel compounds which upgregulate SERCA2 expression is closely linked to the characterization of regulatory sequences of the SERCA2 promoter.

Key Words: Heart failure · Angina pectoris · Metabolism · Fatty acid oxidation · Glucose oxidation · Gene expression

Herz 2002;27:621–36

DOI 10.1007/s00059-002-2428-x

Der Nutzen partieller Hemmer der Fettsäurenoxidation zur metabolischen Therapie von Angina pectoris und Herzinsuffizienz

Hintergrund: Partielle Hemmer der Fettsäurenoxidation sind von großem Interesse, da sie einer dysregulierten Genexpression von hypertrophierten Kardiozyten entgegenwirken können. Einige dieser Verbindungen sind für die Behandlung von Typ-II-Diabetes-mellitus und stabiler Angina pectoris entwickelt worden. Eine Verschiebung von Fettsäurenoxidation zu Glucoseoxidation führt zu einer verringerten Glukoneogenese und verbesserten Ökonomie der Herzarbeit. Die Glucoseoxidation wird von folgenden Enzymhemmern gesteigert: Etomoxir, Oxfenicin, Methylpalmoxirat, S-15176, Metoprolol, Amiodaron, Perhexilin (Carnitinpalmitoyltransferase-1); Aminocarnitin, Perhexilin (Carnitinpalmitoyltransferase-2); Hydrazonopropionsäure (Carnitin-acylcarnitintranslocase); MET-88 (Gamma-Butyrobetainhydroxylase); 4-Bromocrotonsäure, Trimetazidin, möglicherweise Ranolazin (Thiolasen); Hypoglycin (Butyryl-CoA Dehydrogenasen); Dichloracetat (Pyruvatdehydrogenasekinase).

Klinische Studien mit Trimetazidin und Ranolazin haben gezeigt, dass diese Verschiebung im Energiestoffwechsel eine antianginöse Wirkung hat. Etomoxir und MET-88 verbesserten die Funktion überbelasteter Herzen durch Steigerung der Dichte der Ca^{2+}-Pumpe von sarkoplasmatischem Retikulum (SERCA2). Der Promoter von SERCA2 und der Alpha-Myosin schweren Kette hat Sequenzen, die auf Transkriptions-

[1] Molecular Cardiology Laboratory, Department of Internal Medicine and Cardiology, University of Marburg, Germany,
[2] Laboratorio de Biología Molecular, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, México.

faktoren ansprechen, welche von Glucosemetaboliten und/oder PPARalpha-Agonisten beeinflusst werden. Ein weiterer Fortschritt bei der Entwicklung von Substanzen, welche die SERCA2-Expression verstärken können, ist eng an die Aufklärung der regulatorischen Sequenzen des SERCA2-Promoters geknüpft.

Schlüsselwörter: Herzinsuffizienz · Angina pectoris · Stoffwechsel · Fettsäureoxidation · Glucoseoxidation · Genexpression

Introduction

Cardiac metabolism of glucose and fatty acids has been studied primarily from the viewpoint of energy production for contraction and ion homeostasis. Although the main energy supply is provided by the oxidation of fatty acids, the heart can consume also glucose and lactate. Since the use of glucose is associated with a lower oxygen consumption, the possibility of altering the ratio of consumed fatty acids and glucose has attracted interest in the last decades. In particular, the question has been addressed whether a shift in fuel utilization in favor of glucose could be advantageous for the recovery of pump function after ischemic events. The concept of economizing cardiac energetics by increasing glucose utilization was also the basis for the development of drugs which might be useful in angina pectoris. Ideally, drug interventions should reduce the oxygen consumption while the energy production can be maintained. Such drugs are expected to avoid excess energy expenditure associated with fatty acid oxidation. The exact amount of the additional oxygen consumption arising from fatty acid oxidation remains controversial. Various investigators have observed an oxygen wasting effect that is too large to be explained by the different ATP-to-oxygen ratios of these substrates [57]. It was concluded that two different mechanisms are responsible for the oxygen-wasting effect, one that depends on mitochondrial fatty acid oxidation and another that is not affected by an inhibition of this pathway [57].

During our screening studies on drugs which increase the expression of the myosin isoform V1 and sarcoplasmic reticulum Ca^{2+} uptake, we observed that interventions which increase glucose utilization have a potent action on myosin heavy-chain expression [108]. Although these findings might have been unexpected, they can be rationalized in view of the apparent need of the cardiocyte to match gene expression of key proteins with the energetic status. Thus expression of myosin V1 which has a higher energy consumption than myosin V3 could be expected if the higher energy requirements are signaled by an anabolic state. In accordance with this concept is the finding that the catabolic state of fasting [87] reduces myosin V1 [112]. In the present review, compounds are described which can shift cardiac fuel utilization in favor of glucose. If available, potential effects in heart disease beyond coronary artery disease will be discussed. It is hoped that this review will strengthen efforts in elucidating the potential use of antianginal compounds for changing the gene expression of cardiocytes in a manner which is associated with an improved function.

Metabolic Therapy for Overloaded Cardiocytes

It might be argued that overloaded hearts are energy-starved [63] and benefit from the improved energy balance of an increased glucose oxidation and a reduced fatty acid oxidation. This argument would be in accordance with the findings that such drugs have proved promising in patients with stable angina pectoris [127]. Although ATP deprivation can contribute to heart failure [89], it appears unlikely that it can account for the progression of heart failure. Rather, the structural deterioration of heart muscle has a crucial role in the progressive impairment of heart failure whereby a consecutive ventricular dilatation further deteriorates the energetic status. Standard therapy is targeted at reducing an overload and counteracting neuro-endocrine activation resulting from an impaired heart performance. Despite the progress made in the treatment of congestive heart failure, the mortality remains high. It appears, therefore, that potential drug targets have been missed in the current therapy. Of particular importance are in this respect agents which interfere with a dysregulated phenotype of the cardiocyte. Such a drug approach is expected to slow or prevent progression of heart failure.

Progress in this field has been slow because the functional consequences of an altered phenotype have often been rated solely on subcellular features and not on the overall performance of an overloaded heart. It has been argued that a lower myosin V1 expression of

an overloaded heart is beneficial because it results in an improved economy of force generation. This argument does not take into account that a switch in myosin heavy-chain expression is often not a unique event but is associated with an altered expression of numerous other genes. It was, therefore, an unexpected finding that metabolically active compounds which increase myosin V1 are associated with an improved pump function of an overloaded heart. It turned out that an increased myosin V1 proportion can be associated with an enhanced activity of the sarco-(endo-)plasmic reticulum Ca^{2+}-ATPase-2 (SERCA2) [113]. The search for drugs which increase myosin V1 has also been neglected because it has been assumed that human heart does not exhibit myosin V1. This view has, however, been proven incorrect in a number of studies and it has been concluded that the loss of the small proportion of myosin V1 is an adverse event in human hearts [86]. A further hindrance for the development of drugs which interfere with a dysregulated phenotype of pressure overloaded hypertrophied hearts arises from the fact that they do not acutely improve heart performance.

Energy Metabolism of Overloaded Hearts

The normal heart derives approximately 60–80% of energy consumed from fatty acids and the rest from glucose and lactate permitting the use of partial fatty acid oxidation inhibitors [128, 131, 148]. Under conditions of an increased energy demand, the utilization of glucose can markedly increase. In an isovolumically beating heart preparation [69], the consumption of glucose can be increased up to 50% [68, 70]. Although the mechanisms responsible for this shift in fuel metabolism could be related to the high adrenergic drive, it appears that this heart preparation can partially mimic the energetic status of a chronically overloaded heart.

It was an intriguing finding that in the chronically overloaded heart, glucose oxidation is increased [16, 21, 39, 75, 102, 104, 132]. In hypertrophied ischemic ventricle after myocardial infarction [129], a repression of genes which are responsible for the oxidation of fatty acids was observed. To this group belongs the enoyl-CoA isomerase, dienoyl-CoA reductase, hydroxyacyl-CoA dehydrogenase, acyl-CoA synthase and ketoacyl-CoA thiolase [129]. This coordinated repression of enzymes involved in beta-oxidation [115] would be in accordance with a reduced fatty acid utilization. Furthermore, the lipoproteinlipase and CD36, a fatty acid transporter, were repressed [129]. The expression of genes involved in fatty acid oxidation are modulated by the transcription factor PPARalpha. Since PPARalpha was reduced as a consequence of pressure overload [114], the characteristic shift in fuel metabolism has been attributed to a reduced influence of PPARalpha [6].

The DNA chip data on PPARalpha regulated genes provide also a further interpretation of our studies on etomoxir which has a selective influence on the protein phenotype of hypertrophied cardiomyocytes. Etomoxir was developed as an inhibitor of the mitochondrial carnitine palmitoyltransferase-1 (CPT-1) located on the outer mitochondrial membrane (Figure 1). The activity of CPT-1 determines the rate of the mitochondrial uptake of long-chain fatty acids. A CPT-1 inhibition alone would solely reduce the already diminished fatty acid oxidation of pressure overloaded hearts. Since etomoxir had a protective action on the ischemia/reperfusion injury of the kidney similarly to an established PPARalpha agonist [98], the question arises whether the functional improvements due to etomoxir treatment are due to CPT-1 inhibition or some additional yet undefined influences related to PPARalpha activation [156].

CPT-1 Inhibitors

Etomoxir

Etomoxir has been developed for treating non-insulin-dependent diabetes mellitus [54, 147]. This CPT-1 inhibitor has no acute cardiovasuclar effects in rats as shown by an unaltered heart rate (Figure 2) and blood pressure (not shown). Thus, the shift in substrate oxidation in favor of glucose is not counteracted by sympathetic activation. Also in patients with heart failure, no acute effects on hemodynamic parameters were observed [117]. Etomoxir increased the functional recovery of fatty acid perfused ischemic rat hearts which was unrelated to changes in levels of long-chain acylcarnitines and was attributed to an increased glucose use [79]. A chronic treatment of rats with etomoxir increased the SR Ca^{2+}-ATPase activity [109], the Ca^{2+} uptake rate [113], the number of active Ca^{2+} pumps E~P [111, 142], the SERCA2 protein [111, 142] and the SERCA2 mRNA abundance [158] of the heart. In parallel with the effect on SERCA2, the proportion of myosin V1 (2 alpha-MHC) was increased which demonstrates a coordinated expression of genes required for fast relaxation and contraction. At a low dosage, etomoxir had a selective influence on the rate of contraction and relaxation of overloaded hearts [136].

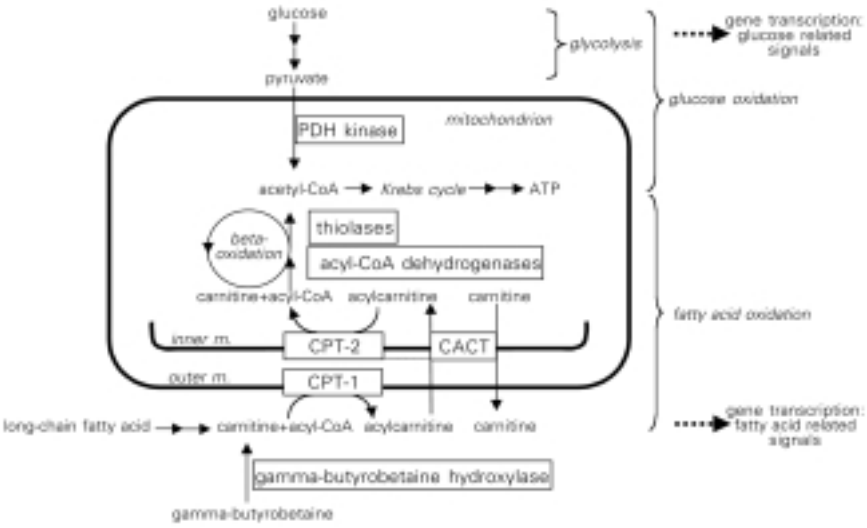

Figure 1. Schematic representation of potential drug targets for reducing cardiac fatty acid oxidation and increasing glucose oxidation (PDH: pyruvate dehydrogenase; CPT: carnitine palmitoyltransferase; CACT: carnitine-acylcarnitine translocase). Only long-chain fatty acids need to be transformed into acylcarnitines before they can enter mitochondria. Medium-chain fatty acids can bypass a CPT-1 block by entering mitochondria directly.

Abbildung 1. Schematische Darstellung von möglichen therapeutischen Ansätzen zur Verringerung einer kardialen Fettsäurenoxidation und einer gesteigerten Glucoseoxidation (PDH: Pyruvatdehydrogenase; CPT: Carnitinpalmitoyltransferase; CACT: Carnitin-Acylcarnitintranslocase). Nur langkettige Fettsäuren müssen in Acylcarnitin umgewandelt werden, bevor sie die innere Mitochondrienmembran passieren können. Mittelkettige Fettsäuren können einen CPT-1-Block umgehen, da sie direkt die Mitochondrienmembran passieren können.

Although it is well accepted that etomoxir inhibits CPT-1, it remains unclear whether additional influences arising from PPARalpha activation have to be considered. Oxirane compounds such as etomoxir can bind and activate PPARalpha [41, 98]. To examine putative additional influences, the CPT-1 inhibition can be bypassed by feeding etomoxir treated rats a medium-chain fatty acid diet containing nonanoate and decanoate [111]. Medium-chain fatty acids do not require CPT-1 for their entry into mitochondria. A medium-chain fatty acid diet is thus expected to result in an unaltered fatty acid oxidation although CPT-1 is inhibited by etomoxir. In rats treated with etomoxir and fed a regular long-chain fatty acid containing diet, the lipid droplet number was moderately increased which was greatly prevented by exchanging the dietary long-chain fatty acids for medium-chain fatty acids [111] (Figure 3). The lipid droplet number was only not significantly increased corresponding to 28.6% of the increase observed in etomoxir treated rats fed a regular diet. The high dose etomoxir treatment resulted in a harmonious ventricular growth (+21% left ventricle, +17% right ventricle [111]) of the heart which, based on the observed phenotype, resembles that of swim-exercised hearts [107]. The cardiac growth was reduced by the medium-chain fatty acid diet similarly to the lipid droplet number (Figure 3). Also the etomoxir-induced decrease in serum triglycerides was prevented by the medium-chain fatty acid diet.

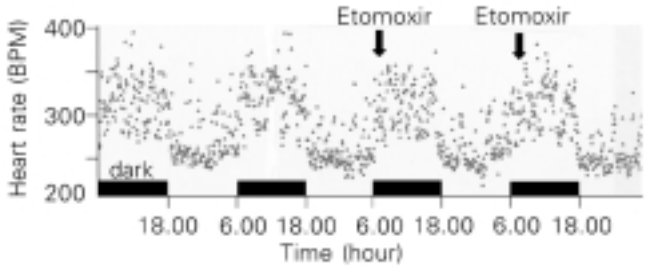

Figure 2. Radiotelemetric monitoring of heart rate of a representative Wistar rat given twice 50 mg/kg racemic etomoxir by gavage. The dark periods are marked which lead to a high motility associated with an increased heart rate. Systolic and diastolic blood pressure was also not significantly affected by etomoxir (not shown).

Abbildung 2. Radiotelemetrische Registrierung der Herzfrequenz einer repräsentativen Wistarratte, der zweimal 50 mg/kg racemisches Etomoxir mit der Schlundsonde verabreicht wurde. Die Dunkelphasen führen zu einer hohen Motilität und einer gesteigerten Herzfrequenz. Der systolische und diastolische Blutdruck wurde durch Etomoxir nicht signifikant beeinflusst (nicht dargestellt).

Etomoxir increased the number of active E~P Ca^{2+} pumps of SR which was prevented to a similar extent as the lipid droplet number (see Figure 3). The SR phospholamban content was not affected by etomoxir. SR Ca^{2+} uptake of ventricular homogenates was increased in etomoxir treated rats irrespective of the presence of the SR Ca^{2+} release inhibitor ruthenium red or the catalytic subunit of protein kinase A which phosphorylates phospholamban. The medium-chain fatty acid diet resulted in SR Ca^{2+} uptake rates that were in between those of etomoxir-treated and untreated rats [111]. Since etomoxir increased SR Ca^{2+} pumps but not phospholamban, Ca^{2+} pumps can be inferred which are not inhibited by dephosphorylated phospholamban leading thereby to a higher overall SR Ca^{2+} uptake rate [141]. In contrast to the E~P Ca^{2+} pumps, the etomoxir-induced

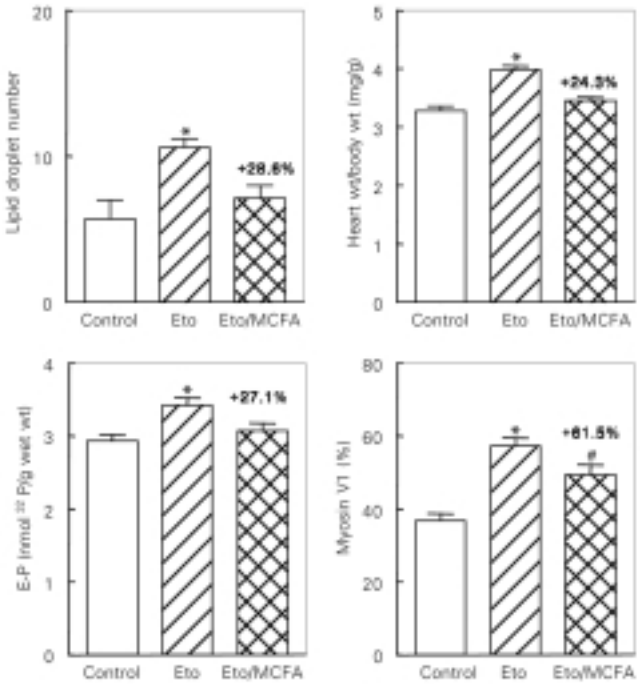

Figure 3. Comparative analysis of the effect of a medium-chain fatty acid diet on etomoxir-induced changes in cardiac lipid droplet number, the heart/body weight ratio, phosphorylated intermediate E~P of the SR Ca^{2+}-ATPase and the myosin V1 proportion. The percentage values given on the bars of the etomoxir treated WKY rats fed a medium-chain fatty acid diet refer to the increase seen in etomoxir treated rats fed a regular long-chain fatty acid diet (Eto: etomoxir treated rats fed a regular long-chain fatty acid diet; Eto/MCFA: etomoxir treated rats fed a medium-chain fatty acid diet [50–65% C9:0, 30–40% C10:0]; *p < 0.05 vs control; #p < 0.05 vs etomoxir treated fed a regular diet). Data are adapted from Rupp et al [111].

Abbildung 3. Vergleichende Untersuchung der Wirkung einer mittelkettigen Fettsäurendiät auf durch Etomoxir verursachte Veränderungen in der Zahl von kardialen Lipidtröpfchen, das Herzgewicht/Körpergewicht-Verhältnis, die phosphorylierte aktive Ca^{2+}-Pumpe (E~P) des sarkoplasmatischen Retikulums (SR) und das Myosin-V1-Isoenzym. Die Prozentzahlen über den Säulen von Etomoxir-behandelten Ratten, die mit mittelkettigen Fettsäuren gefüttert wurden, beziehen sich auf Werte von Etomoxir-behandelten und mit einer langkettigen Fettsäurendiät gefütterten Ratten (Eto: Etomoxir behandelte Ratten, die mit einer langkettigen Fettsäurendiät gefüttert wurden; Eto/MCFA: Etomoxir-behandelte Ratten, die mit einer mittelkettigen (50–65% C9:0, 30–40% C10:0) Fettsäurendiät gefüttert wurden; *p < 0,05 gegen Kontrollen; #p < 0,05 gegen Etomoxir-behandelte Ratten, die mit einer langkettigen Fettsäurendiät gefüttert wurden). Daten nach Rupp et al. [111].

increase in myosin V1 was prevented to a lesser extent (see Figure 3) suggesting that superimposed on CPT-1 inhibition additional influences occur. The myosin V1 proportion was 61.5% of the increase seen in etomoxir treated rats fed the regular diet.

Additional mechanisms can be inferred also from the observation that – although etomoxir just amplifies the effects of pressure overload on cardiac metabolism – it has an opposite action on SERCA2 and myosin. If one postulates that the reduced PPARalpha expression of pressure overloaded hearts results in an impaired transcription also of genes not involved in metabolism, the etomoxir treatment could restore the expression of PPARalpha target genes. Such an approach would differ from a pure PPARalpha agonist treatment which has been reported to be unfavorable in overloaded hearts [153]. Etomoxir would be expected to stimulate the expression of PPARalpha target genes but because of the CPT-1 inhibition, would not increase fatty acid oxidation (Figure 4).

Oxfenicine

CPT-1 inhibitors have been developed for treating non-insulin-dependent diabetes mellitus which is characterized by elevated fatty acid levels. Inhibition of fatty acid oxidation is associated with reduced hyperglycemia due to inhibition of glucose production [35, 40]. Most of the investigational CPT-1 inhibitors have been shown to cause hypoglycemia and some also cause hypoketonemia [121]. Another well characterized CPT-1 inhibitor is oxfenicine (S-2-(4-hydroxyphenyl)glycine, HPG) which is transaminated to the active metabolite 4-hydroxyphenylglyoxylate [130]. In dogs, oxfenicine increased the glucose oxidation from 17 to 40% of total substrate oxidized [33]. In case of high circulating FFA levels, glucose oxidation was increased from 9 to 32%. Also after cardiac denervation which inhibits glycolysis, glucose oxidation was increased from 5 to 24%. It was calculated that the efficiency of the myocardial energy supply is improved by increasing myocardial oxidative carbohydrate utilization [60]. The pharmacologically induced shift in cardiac metabolism may, therefore, be favorable in circumstances with limited oxygen supply [13, 74] or increased oxygen demand [122]. Furthermore, oxfenicine reduced the accumulation of long-chain acylcarnitine in the ischemic myocardium after coronary artery occlusion, whereas the lowering of long-chain acyl-CoA was less pronounced [143]. The myocardial infarct size was also reduced after oxfenicine treatment [143]. As in the case of etomoxir, cardiac hypertrophy can be induced with oxfenicine [46]. The increase in heart weight was due to uniform myocardial fiber hypertrophy involving all cardiac chambers. Although intracellular lipid was increased, vacuolated lysosomal structures were observed only occasionally [46]. It thus appears that also in the case of oxfenicine, the cardiac hypertrophy is not associated with an adverse restruc-

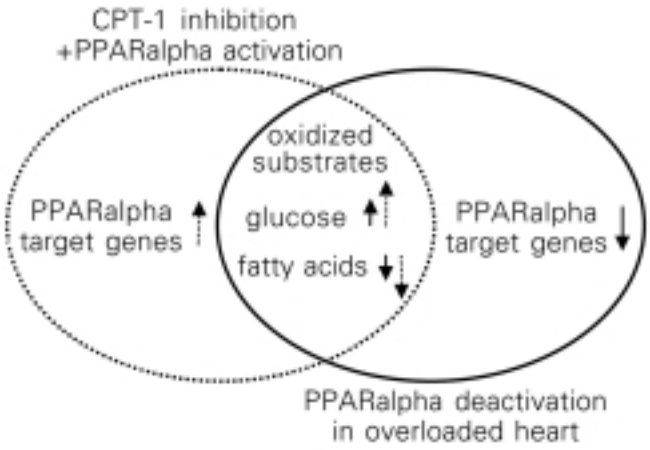

Figure 4. Schematic representation of synergistic effects of etomoxir and pressure overload on cardiac substrate oxidation. By contrast, etomoxir is expected to upregulate PPARalpha target genes while a pressure overload reduces their expression. Contrary to a pure PPARalpha agonist, etomoxir does not increase fatty acid oxidation.

Abbildung 4. Schematische Darstellung synergistischer Wirkungen von Etomoxir und Druckbelastung auf die kardiale Substratoxidation. Bei Etomoxir wird erwartet, dass die Transkription von PPARalpha-Zielgenen gesteigert wird, während eine Druckbelastung die Expression solcher Gene vermindert. Im Gegensatz zu einem reinen PPARalpha-Agonisten verstärkt Etomoxir nicht die Fettsäurenoxidation.

turing characteristic of pressure overloaded hearts. It is in this respect an intriguing finding that the oxidation of palmitate through CPT-1 is involved in the initiation of apoptosis in cardiocytes [73]. Oxfenicine significantly blocked cell death induced by the combination of palmitate and carnitine [73].

2-Tetradecylglycidic Acid

Methyl palmoxirate or 2-tetradecylglycidic acid (TDGA) appears to have a similar profile as oxfenicine with respect to its cardiac effects. Induction of myocardial hypertrophy with methyl palmoxirate retarded the process of ventricular dilatation and produced beneficial effects on systolic function after large myocardial infarction in rats [78]. It was argued that an inadequate hypertrophy of residual myocardium after infarction may contribute to ventricular dilatation and the development of congestive heart failure [78]. It was not taken into account that methyl palmoxirate might change gene expression of cardiocytes as in the case of etomoxir and thereby improve heart function [47].

Trimetazidine Derivative S-15176

The trimetazidine derivative S-15176 protected mitochondria against adverse effects of ischemia reperfusion [36]. It is noteworthy that this compound had a greater inhibitory action on the CPT-1 in the heart (IC_{50} = 17 µM) than in the liver (IC_{50} = 51 µM). In the heart, it was, however, less effective than the physiological inhibitor malonyl-CoA (IC_{50} = 2 µM). It was more potent than amiodarone (IC_{50} = 140 µM). Kinetic experiments demonstrated a non-competitive inhibition of CPT-1 by S-15176 indicating that it did not share with malonyl-CoA the same site of action. In view of findings that adverse effects of CPT-1 inhibition might be related to inhibition of the liver isoform of CPT-1, the S-15176 compound demonstrates that a more cardioselective CPT-1 inhibitor can be developed.

CPT-2 Inhibitors

Aminocarnitine is a potent competitive inhibitor for CPT-1 and CPT-2 (IC_{50} for CPT-2 = 805 nM) [116]. Aminocarnitine resulted in glycogen depletion and accumulation of long-chain acylcarnitine in the heart [56]. Since the long-chain acylcarnitine accumulation was inhibited by the simultaneous addition of etomoxir, it was concluded that aminocarnitine is a specific inhibitor of the inner membrane CPT-2 [56] (see Figure 1). It was an intriguing finding that rats treated for 3 weeks with aminocarnitine did not show cardiac hypertrophy [55]. This contrasted with the effects of etomoxir. As CPT-1 and CPT-2 are both required for the oxidation of long-chain fatty acids in mitochondria, it was concluded that inhibition of fatty acid oxidation per se is not responsible for cell growth, but rather the accumulation of a metabolite, probably long-chain acyl-CoA [55]. Since differences among the carnitine-binding sites on carnitine acyl-transferases exist, selective inhibitors could be designed [116].

Carnitine-Acylcarnitine Translocase Inhibitors

Fatty acid utilization can be reduced also by inhibiting the mitochondrial free carnitine-esterified carnitine exchange across the inner mitochondrial membrane by carnitine acylcarnitine translocase (see Figure 1). The respective hypoglycaemic compounds also reduce fatty acid oxidation [12]. The rate of carnitine uptake in mitochondria was reduced by 2-(3-methyl-cinnamyl-hydrazono-)propionate and 2-(3-phenylpropoxyimino-)butyric acid (BM 13.677) [11]. BM 13.907 which appears to induce also glucose transporter translocation [90] increased cardiac hypertrophy of overloaded hearts and reduced myosin V1 in parallel with the cardiac growth [110]. The reduction of SR Ca^{2+}-ATPase activity of pres-

sure overloaded hearts was, however, prevented by BM 13.907 [110]. Recently it was shown that medium and long-chain (+)-acylcarnitines do not inhibit CPT-1 or CPT-2 but suppress mitochondrial fatty acid transport solely through the inhibition of the carnitine-acylcarnitine translocase [4].

Gamma-Butyrobetaine Hydroxylase Inhibitors

In an alternative approach, the uptake of long-chain fatty acids into mitochondria can be inhibited by reducing the level of carnitine which is required by CPT-1 (see Figure 1). Carnitine is derived from dietary sources and is synthesized in the body. The carnitine synthesis can competitively be inhibited by 3-(2,2,2-trimethylhydrazine-)propionate which is a structural analogue of gamma-butyrobetaine. This agent has also been termed THP, mildronate and MET-88. The decreased carnitine content in the heart was associated with a reduced oxidation of palmitate [123]. MET-88 stimulated the carnitine-independent oxidation of medium-chain fatty acids such as octanoate which appears to occur as a compensation for inhibition of the carnitine-dependent oxidation [59]. MET-88 exhibited various cardioprotective effects. It prevented a decrease of ATP and accumulation of AMP under the influence of isoproterenol [124, 125]. Also the reduction in the activities of mitochondrial electron-transport systems was partially prevented [48]. MET-88 protected against cardiac dysfunction in ischemia/reperfusion preventing the accumulation of long-chain acylcarnitine [2, 51]. MET-88 attenuated the derangement of energy metabolism in the ischemic myocardium without affecting the energy metabolism in the non-ischemic myocardium [67].

It is important to note that MET-88 exhibited effects similar to etomoxir on the function and biochemical parameters of overloaded hearts. MET-88 was examined in rats with congestive heart failure due to myocardial infarction. MET-88 prolonged survival and prevented the rise in right atrial pressure and left ventricular dilatation [50]. The infarcted area was reduced from 30 to 19% [58]. MET-88 attenuated also left ventricular dysfunction in rats with an aorto-caval shunt [88].

The improved function of the overloaded hearts treated with MET-88 was attributed to an increased SR Ca^{2+} pump activity [49, 152]. In left ventricular myocardial homogenates, the SERCA2 protein content was 32% lower in the myocardial infarction group than in the control group [152]. However, in the MET-88 group with myocardial infarction, the SERCA2 content was the same as in the control group [152]. Untreated rats revealed a decrease in the Vmax for SR Ca^{2+} uptake activity which was prevented by MET-88 [49]. The treatment also improved myocardial high-energy phosphate [49]. MET-88 had a greater protective action than a substitution therapy with carnitine [101]. In a pilot clinical trial, the drug exhibited an antiarrhythmic action and resulted in an enhanced physical performance while no major side effects were apparent [92].

Inhibitors of Mitochondrial Fatty Acid Beta-Oxidation

Investigational Agents

Fatty acid oxidation is controlled by the acetyl-CoA/CoASH ratio which is determined by the rate of the citric acid cycle and consequently by the energy demand of the tissue [93, 140]. Degradation of palmitic acid and longer-chain fatty acids is initiated by the beta-oxidation system of the inner membrane while fatty acids shorter than palmitic acid can be oxidized to a certain degree by the matrix system alone [77]. The thiolase-catalyzed step is rate-limiting in beta-oxidation [94]. Inhibitors of mitochondrial acetoacetyl-CoA thiolase, long-chain thiolase and 3-ketoacyl-CoA thiolase (see Figure 1) include 4-pentenoic acid, 2-bromooctanoic acid, 4-bromocrotonic acid [121] and the recent 4-bromotiglic acid [77]. It was concluded that methyl palmoxirate is the compound of choice for experimentally inhibiting mitochondrial uptake of fatty acids and thereby their oxidation, whereas 4-bromocrotonic acid appeared as the best irreversible inhibitor of beta-oxidation [121]. It should be noted that while 4-bromocrotonic acid was converted to its CoA thioester also by heart mitochondria, 4-bromo-2-octenoic acid and valproic acid were activated only by liver mitochondria [151].

Trimetazidine

Trimetazidine (2,3,4-trimethoxybenzyl-piperazine dihydrochloride) has been developed as an antianginal agent. Since it inhibits long-chain fatty acid oxidation, it was examined whether it inhibits CPT-1. Trimetazidine had an inhibitory action (IC_{50} = 1.3 mM) but was less potent than perhexiline (IC_{50} = 77 µM) or amiodarone (IC_{50} = 228 µM) [64]. It was concluded that the relatively low potency of trimetazidine as a CPT-1 inhibitor makes this an unlikely mechanism to explain its therapeutic anti-ischemic effect [64]. Recently it was shown that trimetazidine inhibits long-chain 3-ketoacyl-CoA thiolase (IC_{50} ~ 50 nM) [62]. Trimetazidine is efficient in

protecting isolated cardiac myocytes against the functional alterations induced by substrate-free hypoxia and resulted in a better recovery upon reoxygenation [38]. Trimetazidine exhibited also a protective action in cardiomyopathic hamsters. Long-term oral treatment was more efficient than the Ca^{2+} blocker verapamil [25]. Trimetazidine increased the median survival time by 57%, cardiac hypertrophy was reduced and the total Ca^{2+} level reverted to that of normal hamsters [25]. This finding would be in accordance with studies showing that cardiomyopathic hamsters exhibit a reduced pyruvate dehydrogenase activity [28].

Trimetazidine has been examined in several trials as an antianginal agent, both as monotherapy and combined with "classical" anti-ischemic compounds [9]. When compared with nifedipine, trimetazidine was similar in reducing the number of anginal attacks and in raising the ischemic threshold in patients with stable angina [26]. However, the rate-pressure product at the same workload decreased with nifedipine and remained unchanged with trimetazidine [26]. Also patients with stable angina uncontrolled with diltiazem exhibited an antianginal action after combination treatment with trimetazidine [82]. In the Trimetazidine European Multicenter Study, trimetazidine was compared with propranolol in patients with stable angina pectoris [27]. Exercise duration was increased and the number of anginal attacks was reduced equally by both drugs. Trimetazidine did again not reduce the rate pressure product [27]. It appears that trimetazidine improves also the ejection fraction in patients with severe ischemic cardiomyopathy [20]. Trimetazidine improved resting left ventricular function and the severity of dobutamine-induced ischemic myocardial dysfunction [9, 10, 80]. In patients with chronic heart failure of NYHA functional class II–III, various hemostatic and biochemical blood parameters were improved [134].

Trimetazidine is generally well tolerated and only minor side effects have been reported (drowsiness, sedation, diarrhea) [9]. The lack of major adverse effects is important since it shows that a shift in cardiac metabolism in favor of glucose represents a realistic drug target. Unfortunately, data similar to etomoxir and MET-88 on the function of overloaded cardiocytes are currently not available with trimetazidine.

Ranolazine

Ranolazine (RS 43285) has exhibited an antianginal efficacy in humans and anti-ischemic activity in animals [85]. Direct activation of pyruvate dehydrogenase and stimulation of glucose oxidation could account for the anti-ischemic effects. Studies failed, however, to demonstrate any effects of ranolazine on pyruvate dehydrogenase kinase or phosphatase, or on pyruvate dehydrogenase catalytic activity, suggesting that ranolazine activates pyruvate dehydrogenase indirectly. Ranolazine reduced the acetyl-CoA content resulting from an inhibition of fatty acid beta-oxidation leading to activation of pyruvate dehydrogenase [22]. In reperfused ischemic working hearts, ranolazine improved the functional outcome, which was associated with significant increase in glucose oxidation, a reversal of the increased fatty acid oxidation and a smaller but significant increase in glycolysis [84]. Ranolazine reduced the myocardial infarct size of 33% in rats after coronary artery occlusion [155]. The infusion of ranolazine also attenuated the release of cardiac troponin T [155]. However, no protection from injury to regionally ischemic and reperfused dog myocardium was observed [18].

In several phase III clinical studies, ranolazine has been shown to improve exercise-induced myocardial ischaemia and to reduce the severity of angina pectoris [118]. The antianginal activity of ranolazine was assessed in patients with chronic stable angina pectoris who remained symptomatic despite treatment with a beta blocker or diltiazem. A significant improvement was observed in exercise duration, in time to angina, and ST-segment depression. Both, heart rate and arterial pressure were unchanged after ranolazine [23]. In a trial including patients with chronic stable angina, all exercise parameters improved [97]. It was concluded that immediate-release ranolazine is effective and well tolerated. However, this short-acting formulation would not be adequate for continuous protection. Either larger or more frequent doses or a sustained-release formulation would be required [97]. It has, however, also been reported that a therapy with ranolazine was not superior to placebo [135]. To assess effects of ranolazine in patients with previous transmural myocardial infarction, left ventricular hemodynamic and angiographic data were obtained before and after intravenous infusion of ranolazine [52]. In the ischemic segments, the administration of ranolazine significantly increased the regional peak filling rate and regional wall lengthening during the isovolumic relaxation period. It was concluded that ranolazine improves diastolic function of the non-infarcted myocardium under chronic ischemic conditions [52].

Butyryl-CoA Dehydrogenase Inhibitors

The fatty acid beta-oxdation can be reduced also by inhibiting butyryl-CoA dehydrogenase as exemplified by the experimental compound hypoglycin [121] (see Figure 1). Salicylate at high doses also decreased beta-oxidation in fibroblasts by reversible inhibition of long-chain 3-hydroxyacyl-CoA dehydrogenase activity [43, 81]. In this context it should be pointed out that an excessive inhibition of fatty oxidation has detrimental consequences which include hypoglycaemia due to impaired gluconeogenesis, accumulation of fatty acids, fatty acyl-CoAs, and acylcarnitines with depletion of free CoA and carnitine [95]. The accumulated products can further damage mitochondria, uncouple oxidative phosphorylation or increase mitochondrial permeability leading to mitochondrial swelling and steatosis particularly of hepatic cells [95].

Pyruvate Dehydrogenase Activators

Dichloroacetate activates pyruvate dehydrogenase, the rate-limiting enzyme of glucose oxidation and thus preferentially facilitates aerobic oxidation of carbohydrate over fatty acids [14, 126] (see Figure 1). Dichloroactetate is a PDHa kinase inhibitor and thereby activates pyruvate dehydrogenease [22]. Dichloroacetate, given at the time of reperfusion, normalized postischemic function of hypertrophied rat hearts and improved the coupling between glucose oxidation and glycolysis [145]. Treatment with dichloroacetate did not alter the infarct size [45]. In patients with NYHA functional class III–IV congestive heart failure, a dichloroacetate administration for 30 minutes stimulated myocardial lactate consumption and improved left ventricular mechanical efficiency. Stroke volume and left ventricular minute work increased with a simultaneous reduction in myocardial oxygen consumption [15]. However, an intravenous infusion of the same dose dichloroacetate over 15 minutes in patients with heart failure and ejection fraction < or = 40% was not associated with improvement in non-invasively assessed left ventricular function [76]. Although no data are available whether dichloroacetate affects gene expression of an overloaded cardiocyte similarly to etomoxir, it should be noted that dichloroacetate reversed the depressed density of the Ca^{2+}-independent, transient outward current Ito in surviving cardiocytes from infarcted rat hearts [105].

Sympathetic Activity and Phosphodiesterase Inhibitors

Sympathetic activity is a major determinant of glucose oxidation [106]. In chronically denervated dog hearts, glucose oxidation was inhibited [30]. The contribution of fatty acid utilization to overall substrate oxidation rose from 31% of controls to 48% of denervated hearts [34]. The content or activity of a set of fatty acid handling proteins did not change, while the active form of pyruvate dehydrogenase declined [139]. It was concluded that patients with transplanted hearts are likely to show myocardial metabolic inefficiency [31, 32].

In contrast to denervation, a raised sympathetic activity is expected to stimulate glucose oxidation. In epinephrine-treated hearts, the contribution of glucose (glycolysis and glucose oxidation) to ATP production increased from 13 to 36%, which was accompanied by a reciprocal decrease in the contribution of fatty acid oxidation to ATP production from 83 to 63% [24]. The increase in glucose oxidation was accompanied by a significant increase in pyruvate dehydrogenase complex activity in the active form [24]. The increased energy demand induced by epinephrine was initially supplied by a burst of glycogenolysis and followed by delayed increase in the use of exogenous glucose (eventually contributing 29% to ATP synthesis) [44]. Thus the heart responds to an acute increase in energy demand by selective oxidation of glycogen [44].

Drugs which raise intracellular cAMP are expected to increase glucose oxidation at the expense of fatty acid oxidation. One of the early agents for which a shift in substrate metabolism was shown is carbocromen. This inhibitor of phosphodiesterase increased the cAMP content of the rat heart by up to 30% [120]. The uptake of palmitic acid was inhibited by up to 40%, while oxygen consumption diminished by about 20%. The uptake of glucose into the heart tissue increased by 30% [72, 119]. The antianginal effects by carbocromen appear, however, to be mediated through its coronary vasodilator action [144] whereby unfavorable effects due to "coronary steal" have to be considered [71].

Metoprolol, Perhexiline and Amiodarone

Various clinically approved drugs appear to affect the balance of energy metabolism by mechanisms which are not related to their primary pharmacological target. Among the drugs with this additional action are metoprolol, perhexiline and amiodarone. In the case of metoprolol, it was examined whether the improved heart

function with beta-blockade in heart failure is associated with an altered CPT-1 activity [96]. In dogs with coronary microembolism-induced heart failure, the progressive decrease in cardiac function was prevented by treatment with metoprolol, as reflected by an improved ejection fraction. Treated dogs had a markedly decreased CPT-1 activity along with an increased triglyceride concentration [96]. It was concluded that the improved function observed with beta blockers in heart failure could be due, in part, to a decrease in CPT-1 activity and less fatty acid oxidation [96].

The question was also addressed whether the antianginal effects of perhexiline and amiodarone involve a shift in cardiac metabolism [66]. Perhexiline produced a concentration-dependent inhibition of CPT-1 in rat cardiac and hepatic mitochondria, with half-maximal inhibition at 77 and 148 µM, respectively. Amiodarone also inhibited cardiac CPT-1. The rank order of potency for inhibition was malonyl-CoA > 4-hydroxyphenylglyoxylate = perhexiline > amiodarone = monohydroxyperhexiline [66]. CPT-1 inhibition by perhexiline was competitive with respect to palmitoyl-CoA but noncompetitive with respect to carnitine [66]. It was concluded that the CPT-1 inhibition is likely to contribute to the anti-ischemic effects of both perhexiline and amiodarone [66]. Perhexiline inhibits, however, also CPT-2 similar (IC_{50} and Emax) to CPT-1 [65]. Thus, myocardial concentrations of long-chain acylcarnitines, products of CPT-1 action, were decreased by oxfenicine and unaffected by perhexiline. Perhexiline inhibited myocardial release of lactate during normal flow. Perhexiline protected also against diastolic dysfunction during low-flow ischemia in the rat heart possibly resulting from simultaneous effects on CPT-1 and CPT-2. [65].

Branched Fatty Acids as Natural PPAR Agonists

Fatty acids are known to reduce glucose oxidation and to stimulate glucose formation via the Randle cycle [100]. By contrast, the branched fatty acid phytanic acid can enhance glucose uptake which was explained by the increase in mRNA expression of glucose transporters-1 and -2 and glucokinase [53]. Phytanic acid is a ligand of the 9-cis-retinoic acid receptor and PPARalpha [37]. It is assumed that phytanic acid serves as a dietary signal molecule that induces the catabolism of fatty acids by activating PPARalpha [37]. Phytanic acid would thus exhibit effects similar to lipid-lowering fibrates which are PPARalpha agonists [42].

Molecular Mechanisms of CPT1 Inhibitors and PPAR Agonists Mediating Transcriptional Changes in Gene Expression

PPARalpha

Influences on gene expression of the cardiocyte can arise from alterations in the level of fatty acids and sugar metabolites. In the heart, as well as in liver and adipose tissue, the expression of several genes encoding mitochondrial fatty acid beta-oxidation enzymes such as CPT-1, fatty acid translocase, fatty acid-binding protein, acyl-CoA synthase, long-chain fatty acyl-CoA dehydrogenase, and UCP-3 are regulated at the transcriptional level by long-chain fatty acids through the binding of PPARs which in turn bind to peroxisome proliferator-responsive elements (PPREs) [8, 154]. PPARs belong to the steroid, thyroid, and retinoid receptor super family of nuclear receptors. Three members of the PPAR family have been identified, PPARalpha, PPARdelta (also called PPARbeta, FFAR or NUC1), and PPARgamma. PPARalpha is mainly expressed in the heart, skeletal muscle, liver, kidney and vascular endothelial cells. During cardiac development and in the hypertrophic and failing heart, the level of expression of genes encoding mitochondrial fatty acid beta-oxidation enzymes is regulated at the transcriptional level [8, 19, 83, 137, 138]. The gene expression of the muscle isoform mCPT-1 is induced by long-chain fatty acids by PPARalpha in the heart [19, 83, 138]. PPARalpha is involved in the control of lipoprotein metabolism, fatty acid oxidation and cellular uptake of fatty acids [17]. The transcriptional activity of PPARalpha is stimulated by insulin, fibrates, phenylacetate and its analogues, and the selective agonists WY14643, JTT-501, GW2331 and PD72953 [17, 29]. Etomoxir is a compound that binds irreversibly to the catalytic site of CPT-1 inhibiting its activity, but also upregulates fatty acid oxidation enzymes [98]. The increased transcription of fatty acid oxidation genes could be due to accumulation of long-chain fatty acids in the cytoplasm. However, because of the chemical structure of etomoxir, it has been suggested to be a ligand for PPARalpha [98]. Etomoxir, in the liver can act as peroxisomal proliferator, increasing DNA synthesis and liver growth. Thus, etomoxir, in addition of being a CPT1 inhibitor could be considered as a PPARalpha agonist.

PPARalpha is downregulated in the hypertrophied heart [6, 61]. It has thus been suggested that the reduced DNA binding activity of PPARalpha may be responsible for the downregulated expression of cardiac fatty

acid oxidation enzyme genes. A decreased PPARalpha expression during the development of cardiac hypertrophy could also contribute to the pathologic remodeling associated with contractile dysfunction of the heart. In addition, as an early response mechanism to cope with the energy requirements during cardiac hypertrophy, p38 activated MAPK phosphorylates PPARalpha thereby activating it. This suggests that PPARalpha is a downstream effector of p38 kinase-dependent stress-activated signaling in cardiomyocytes [7].

PPARgamma

PPARgamma is a key receptor involved in the control of fat metabolism and glucose homeostasis. PPARgamma is expressed mainly in adipose tissue and plays a major role in adipocyte differentiation [5]. It is also expressed in the heart but at lower level than PPARalpha. Thiazolidinediones (troglitazone, rosiglitazone, ciglitazone and pioglitazone), and cyclopentanone prostaglandins 15D-PGJ2 and PGA1 are PPARgamma activators [5]. Upon activation, PPARgamma forms heterodimers with RXR and binds to PPRE activating target genes. In the pressure overloaded hypertrophied heart, treatment with pioglitazone inhibits the development of the hypertrophy [3]. In PPARgamma deficient mice, a pressure overload induced a more prominent heart weight-to-body weight ratio than in wild type mice [3]. In ischemic and infarcted hearts, PPARgamma ligands reduced the tissue necrosis and inhibited the activation of NFkappaB which in turn reduces expression of inducible nitric oxide synthase [146]. Taken together, the current evidence suggests that PPARgamma is involved in inhibition of cardiac hypertrophy and prevention of necrosis in ischemic myocardium, possibly involving NFkappaB pathways.

SERCA2 Promoter Regulatory Sequences

In animal models of cardiac hypertrophy and patients with heart failure, the mRNA expression of SERCA2 is depressed arising from a reduced gene transcription [1, 99, 103, 133]. Treatment with etomoxir increased the SERCA2 mRNA level in animal hearts [158] and in cardiocytes in culture (Vetter & Rupp, Zarain-Herzberg & Rupp; unpublished). The increase in SERCA2 mRNA could be regulated at the transcriptional level. As putative candidates for the etomoxir-induced increase in SERCA2 expression, several sequences of the promoter of the SERCA2 gene have to be considered [157]. Because of the possibility that etomoxir could act as a PPARalpha ligand activator, this receptor is a potential candidate for the transcriptional action of etomoxir. Within 1.0 kb of the human SERCA2 regulatory region there are four regions that share homology with the PPRE consensus sequence 5'-TGAMCT T TGNCCT AGWTYYG-3' [83] (Figure 5). The sequence of these putative PPREs is highly conserved among the human, rabbit, rat and mouse SERCA2 genes. Further work is required to examine whether etomoxir increases the transcription of the SERCA2 gene and if PPARalpha binds to the putative PPREs.

Because etomoxir increases glucose utilization, the concentration of glucose metabolites such as glucose-6-phosphate and/or xylulose-5-phosphate may also increase, in turn activating intracellular signaling mechanisms that may lead to increased transcription of target genes. The glucose response sequences of the L-PK, S14 and fatty acid synthase have been well characterized [137]. Those sequences have the consensus E-box DNA binding site (5'-CANNTG-3') that could bind the upstream stimulatory factors (USFs). USFs are basic helix-loop-helix leucine zipper (bHLHZ) transcription factors. Two USF factors named USF1 and USF2 have been identified. USF1 has been shown to be expressed in the heart and may be a candidate for glucose mediated signaling [137, 157]. The alpha-MHC gene proximal promoter contains an E-box/USF motif (5'-CACGTG-3') near the TATA-box. This E-box/USF motif can bind USF1 and is necessary for high levels of basal transcription. It has been suggested that this motif may function as a glucose response element [91, 150]. USF1 was phosphorylated by protein kinase C and cAMP-dependent

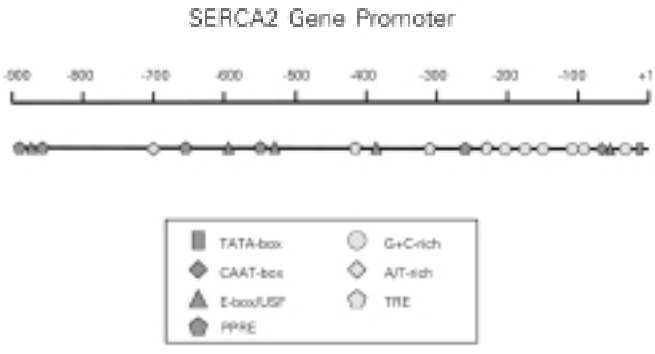

Figure 5. Identification of regulatory sequences within the SERCA2 gene promoter (PPRE: peroxisome proliferator-responsive element; TRE: thyroid hormone-response element).

Abbildung 5. Regulatorische Sequenzen des SERCA2-Gen-Promoters (PPRE: Peroxisome Proliferator-Responsive Element; TRE: Thyroid Hormone-Response Element.

protein kinase [149]. Phosphorylated USF1 had increased DNA binding activity [149]. The proximal SERCA2 promoter also contains a consensus E-box/USF motif (5'-CACATG-3') just upstream from the TATA-box which can be identified as potential glucose response element [157]. There are another three consensus E-box/USF sequences within the 1.5 kb SERCA2 sequence of regulatory region that may function as glucose response elements (see Figure 5).

Taken together, it can be suggested that the transcriptional effects of etomoxir could be due to: 1. shift in energy metabolism with increased glucose utilization and 2. PPARalpha activation [157]. Therefore, etomoxir can be considered as a novel transcriptional modulator that improves the function of diseased hearts, although further experimental work is required to fully understand the mechanism of action of etomoxir and related compounds.

Conclusions

It can be concluded that the improvement in heart function as well as SERCA2 and myosin V1 expression of overloaded hearts observed with etomoxir is not unique for this CPT-1 inhibitor but can be induced also by MET-88 [152]. This gamma-butyrobetaine hydroxylase inhibitor leads to a reduced fatty acid oxidation by inhibiting carnitine synthesis. As in the case of etomoxir, it is however also expected to increase fatty acids in the cytoplasm which could act as PPARalpha agonists.

The clinical trials with trimetazidine and ranolazine demonstrate that a shift in cardiac substrate metabolism is well tolerated. Thus, any adverse events in the case of etomoxir have to be attributed either to the dosage used or the structural properties of the molecule. Since adverse events can arise from the inhibition of the liver isoform of CPT-1, it is important to note that inhibitors can be developed with a higher affinity for the muscle CPT-1 isoform as demonstrated by the trimetazidine derivative S-15176 [36]. Efforts should be made to examine whether clinically approved antianginal drugs such as trimetazidine and ranolazine can induce changes in the cardiocyte phenotype as described for etomoxir and MET-88. Also potentially beneficial effects of lipid-lowering fibrates due to PPARalpha activation on gene expression of overloaded cardiocytes require further examination.

One of the key features of hearts treated with etomoxir is an increased SERCA2 expression. The promoters of SERCA2 and alpha-MHC exhibit sequences which are expected to respond to transcription factors responsive to glucose metabolites and/or PPAR agonists. The transcriptional effects of etomoxir in the heart could be due at least in part to PPARalpha activation in addition to effects arising from CPT-1 inhibition. Further progress in elucidating novel compounds which upregulate SERCA2 expression is closely linked to the characterization of these regulatory sequences of the SERCA2 promoter.

Acknowledgements

The experimental studies were supported by the German Research Foundation (Ru 245/7-1) and from CONACyT (31902-N) and PAPIIT (IN-208599), México.

References

1. Aoyagi T, Yonekura K, Eto Y, et al. The sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) gene promoter activity is decreased in response to severe left ventricular pressure-overload hypertrophy in rat hearts. J Mol Cell Cardiol 1999;31:919–26.
2. Asaka N, Muranaka Y, Kirimoto T, Miyake H. Cardioprotective profile of MET-88, an inhibitor of carnitine synthesis, and insulin during hypoxia in isolated perfused rat hearts. Fundam Clin Pharmacol 1998;12:158–63.
3. Asakawa M, Takano H, Nagai T, et al. Peroxisome proliferator-activated receptor gamma plays a critical role in inhibition of cardiac hypertrophy in vitro and in vivo. Circulation 2002;105:1240–6.
4. Baillet L, Mullur RS, Esser V, McGarry JD. Elucidation of the mechanism by which (+)-acylcarnitines inhibit mitochondrial fatty acid transport. J Biol Chem 2000;275:36766–8.
5. Barbier O, Torra IP, Duguay Y, et al. Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. Arterioscler Thromb Vasc Biol 2002;22:717–26.
6. Barger PM, Brandt JM, Leone TC, Weinheimer CJ, Kelly DP. Deactivation of peroxisome proliferator-activated receptor-alpha during cardiac hypertrophic growth. J Clin Invest 2000;105:1723–30.
7. Barger PM, Browning AC, Garner AN, Kelly DP. p38 mitogen-activated protein kinase activates peroxisome proliferator-activated receptor alpha: a potential role in the cardiac metabolic stress response. J Biol Chem 2001;276:44495–01.
8. Barger PM, Kelly DP. PPAR signaling in the control of cardiac energy metabolism. Trends Cardiovasc Med 2000;10:238–45.
9. Belardinelli R. Trimetazidine and the contractile response of dysfunctional myocardium in ischaemic cardiomyopathy. Rev Port Cardiol 2000;19:Suppl 5:V35–9.
10. Belardinelli R, Purcaro A. Effects of trimetazidine on the contractile response of chronically dysfunctional myocardium to low-dose dobutamine in ischaemic cardiomyopathy. Eur Heart J 2001;22:2164–70.
11. Beneking M, Oellerich M, Binder L, Choitz GF, Haeckel R. Inhibition of mitochondrial carnitine acylcarnitine translocase by hypoglycaemia-inducing substances. J Clin Chem Clin Biochem 1990;28:323–7.
12. Beneking M, Oellerich M, Haeckel R, Binder L. Inhibition of mitochondrial carnitine acylcarnitine translocase-mediated uptake of carnitine by 2-(3-methyl-cinnamyl-hydrazono)-propionate. Hydrazonopropionic acids, a new class of hypoglycaemic substances, VI. J Clin Chem Clin Biochem 1987;25:467–71.

13. Bergman G, Atkinson L, Metcalfe J, Jackson N, Jewitt DE. Beneficial effect of enhanced myocardial carbohydrate utilisation after oxfenicine (L-hydroxyphenylglycine) in angina pectoris. Eur Heart J 1980;1:247–53.
14. Bersin RM, Stacpoole PW. Dichloroacetate as metabolic therapy for myocardial ischemia and failure. Am Heart J 1997;134:841–55.
15. Bersin RM, Wolfe C, Kwasman M, et al. Improved hemodynamic function and mechanical efficiency in congestive heart failure with sodium dichloroacetate. J Am Coll Cardiol 1994;23:1617–24.
16. Bishop SP, Altschuld RA. Increased glycolytic metabolism in cardiac hypertrophy and congestive failure. Am J Physiol 1970;218: 153–9.
17. Bishop-Bailey D. Peroxisome proliferator-activated receptors in the cardiovascular system. Br J Pharmacol 2000;129:823–34.
18. Black SC, Gralinski MR, McCormack JG, Driscoll EM, Lucchesi BR. Effect of ranolazine on infarct size in a canine model of regional myocardial ischemia/reperfusion. J Cardiovasc Pharmacol 1994; 24:921–8.
19. Brandt JM, Djouadi F, Kelly DP. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. J Biol Chem 1998;273:23786–92.
20. Brottier L, Barat JL, Combe C, Boussens B, Bonnet J, Bricaud H. Therapeutic value of a cardioprotective agent in patients with severe ischaemic cardiomyopathy. Eur Heart J 1990;11:207–12.
21. Christe ME, Rodgers RL. Altered glucose and fatty acid oxidation in hearts of the spontaneously hypertensive rat. J Mol Cell Cardiol 1994;26:1371–5.
22. Clarke B, Wyatt KM, McCormack JG. Ranolazine increases active pyruvate dehydrogenase in perfused normoxic rat hearts: evidence for an indirect mechanism. J Mol Cell Cardiol 1996;28: 341–50.
23. Cocco G, Rousseau MF, Bouvy T, et al. Effects of a new metabolic modulator, ranolazine, on exercise tolerance in angina pectoris patients treated with beta-blocker or diltiazem. J Cardiovasc Pharmacol 1992;20:131–8.
24. Collins-Nakai RL, Noseworthy D, Lopaschuk GD. Epinephrine increases ATP production in hearts by preferentially increasing glucose metabolism. Am J Physiol 1994;267:H1862–71.
25. D'hahan N, Taouil K, Dassouli A, Morel JE. Long-term therapy with trimetazidine in cardiomyopathic Syrian hamster BIO 14:6. Eur J Pharmacol 1997;328:163–74.
26. Dalla-Volta S, Maraglino G, Della-Valentina P, Viena P, Desideri A. Comparison of trimetazidine with nifedipine in effort angina: a double-blind, crossover study. Cardiovasc Drugs Ther 1990;4 Suppl 4:853–9.
27. Detry JM, Leclercq PJ. Trimetazidine European Multicenter Study versus propranolol in stable angina pectoris: contribution of Holter electrocardiographic ambulatory monitoring. Am J Cardiol 1995;76:8B–11B.
28. Di Lisa F, Fan CZ, Gambassi G, Hogue BA, Kudryashova I, Hansford RG. Altered pyruvate dehydrogenase control and mitochondrial free Ca^{2+} in hearts of cardiomyopathic hamsters. Am J Physiol 1993;264:H2188–97.
29. Djouadi F, Brandt JM, Weinheimer CJ, Leone TC, Gonzalez FJ, Kelly DP. The role of the peroxisome proliferator-activated receptor alpha (PPAR alpha) in the control of cardiac lipid metabolism. Prostaglandins Leukot Essent Fatty Acids 1999;60:339–43.
30. Drake AJ, Papadoyannis DE, Butcher RG, Stubbs J, Noble MI. Inhibition of glycolysis in the denervated dog heart. Circ Res 1980;47:338–45.
31. Drake-Holland AJ, Cummins P, English TA, Wallwork J, Birch PJ. Metabolic changes in the autotransplanted baboon heart. Transplantation 1984;38:454–9.
32. Drake-Holland AJ, Noble MI. Cellular abnormalities in chronically denervated myocardium. Implications for the transplanted heart. Circulation 1989;80:1476–81.
33. Drake-Holland AJ, Passingham JE. The effect of Oxfenicine on cardiac carbohydrate metabolism in intact dogs. Basic Res Cardiol 1983;78:19–27.
34. Drake-Holland AJ, van der Vusse GJ, Roemen TH, et al. Chronic catecholamine depletion switches myocardium from carbohydrate to lipid utilisation. Cardiovasc Drugs Ther 2001;15:111–7.
35. Eistetter K, Wolf HP. Synthesis and hypoglycemic activity of phenylalkyloxiranecarboxylic acid derivatives. J Med Chem 1982;25:109–13.
36. Elimadi A, Sapena R, Settaf A, Le Louet H, Tillement J, Morin D. Attenuation of liver normothermic ischemia--reperfusion injury by preservation of mitochondrial functions with S-15176, a potent trimetazidine derivative. Biochem Pharmacol 2001;62:509–16.
37. Ellinghaus P, Wolfrum C, Assmann G, Spener F, Seedorf U. Phytanic acid activates the peroxisome proliferator-activated receptor alpha (PPARalpha) in sterol carrier protein 2-/sterol carrier protein x-deficient mice. J Biol Chem 1999;274:2766–72.
38. Fantini E, Athias P, Demaison L, Grynberg A. Protective effects of trimetazidine on hypoxic cardiac myocytes from the rat. Fundam Clin Pharmacol 1997;11:427–39.
39. Feinendegen LE, Henrich MM, Kuikka JT, Thompson KH, Vester EG, Strauer B. Myocardial lipid turnover in dilated cardiomyopathy: a dual in vivo tracer approach. J Nucl Cardiol 1995;2:42–52.
40. Foley JE. Rationale and application of fatty acid oxidation inhibitors in treatment of diabetes mellitus. Diabetes Care 1992;15:773–84.
41. Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc Natl Acad Sci USA 1997;94:4312–7.
42. Fruchart JC. Peroxisome proliferator-activated receptor-alpha activation and high-density lipoprotein metabolism. Am J Cardiol 2001;88:24N–29N.
43. Glasgow JF, Middleton B, Moore R, Gray A, Hill J. The mechanism of inhibition of beta-oxidation by aspirin metabolites in skin fibroblasts from Reye's syndrome patients and controls. Biochim Biophys Acta 1999;1454:115–25.
44. Goodwin GW, Ahmad F, Doenst T, Taegtmeyer H. Energy provision from glycogen, glucose, and fatty acids on adrenergic stimulation of isolated working rat hearts. Am J Physiol 1998;274: H1239–47.
45. Gozal Y, Wolff RA, Van Winkle DM. Manipulations in glycogen metabolism and the failure to influence infarct size in the ischaemic rabbit heart. Eur J Anaesthesiol 2002;19:495–503.
46. Greaves P, Martin J, Michel MC, Mompon P. Cardiac hypertrophy in the dog and rat induced by oxfenicine, an agent which modifies muscle metabolism. Arch Toxicol Suppl 1984;7:488–93.
47. Gunther J, Wagner K, Theres H, et al. Myocardial contractility after infarction and carnitine palmitoyltransferase I inhibition in rats. Eur J Pharmacol 2000;406:123–6.
48. Hanaki Y, Sugiyama S, Ozawa T. Effect of 3-(2,2,2-trimethylhydrazinium) propionate, gamma-butyrobetaine hydroxylase inhibitor, on isoproterenol-induced mitochondrial dysfunction. Res Commun Chem Pathol Pharmacol 1989;64:157–60.
49. Hayashi Y, Ishida H, Hoshiai M, et al. MET-88, a gamma-butyrobetaine hydroxylase inhibitor, improves cardiac SR Ca^{2+} uptake activity in rats with congestive heart failure following myocardial infarction. Mol Cell Biochem 2000;209:39–46.
50. Hayashi Y, Kirimoto T, Asaka N, et al. Beneficial effects of MET-88, a gamma-butyrobetaine hydroxylase inhibitor in rats with heart failure following myocardial infarction. Eur J Pharmacol 2000;395:217–24.

51. Hayashi Y, Tajima K, Kirimoto T, Miyake H, Matsuura N. Cardioprotective effects of MET-88, a gamma-butyrobetaine hydroxylase inhibitor, on cardiac dysfunction induced by ischemia/reperfusion in isolated rat hearts. Pharmacology 2000;61:238–43.
52. Hayashida W, van Eyll C, Rousseau MF, Pouleur H. Effects of ranolazine on left ventricular regional diastolic function in patients with ischemic heart disease. Cardiovasc Drugs Ther 1994;8:741–7.
53. Heim M, Johnson J, Boess F, et al. Phytanic acid, a natural peroxisome proliferator-activated receptor (PPAR) agonist, regulates glucose metabolism in rat primary hepatocytes. FASEB J 2002;16:718–20.
54. Hubinger A, Weikert G, Wolf HP, Gries FA. The effect of etomoxir on insulin sensitivity in type 2 diabetic patients. Horm Metab Res 1992;24:115–8.
55. Hulsmann WC, Peschechera A, Schneijdenberg CT, Verkleij AJ. Comparison of the effects of carnitine palmitoyltransferase-1 and -2 inhibitors on rat heart hypertrophy. Cardioscience 1994;5: 193–7.
56. Hulsmann WC, Schneijdenberg CT, Verkleij AJ. Accumulation and excretion of long-chain acylcarnitine by rat hearts; studies with aminocarnitine. Biochim Biophys Acta 1991;1097:263–9.
57. Hutter JF, Piper HM, Spieckerman PG. Effect of fatty acid oxidation on efficiency of energy production in rat heart. Am J Physiol 1985;249:H723–8.
58. Ianson TM, Bauman VR, Grom NP, Kienkas IA, Kalvin'sh II. Cardioprotective effect of carnitine and its synthetic analog 3-(2,2,2-trimethylhydrazinium) propionate in rats with experimental myocardial infarction. Vopr Med Khim 1988;34:122–5.
59. Kagan TI, Simkhovich BZ, Kalvinysh II, Lukevits EI. Study of the effect of an inhibitor of carnitine-dependent metabolism of mildronate on the oxidation of fatty acids in the liver mitochondria of intact rats. Vopr Med Khim 1991;37:44–6.
60. Kahles H, Schafer W, Lick T, Junggeburth J, Kochsiek K. Changes in myocardial substrate and energy metabolism by S-(4)-hydroxyphenylglycine and an N-(6)-derivative of adenosine. Basic Res Cardiol 1986;81:258–66.
61. Kanda H, Nohara R, Hasegawa K, Kishimoto C, Sasayama S. A nuclear complex containing PPARalpha/RXRalpha is markedly downregulated in the hypertrophied rat left ventricular myocardium with normal systolic function. Heart Vessels 2000; 15:191–6.
62. Kantor PF, Lucien A, Kozak R, Lopaschuk GD. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A thiolase. Circ Res 2000;86:580–8.
63. Katz AM. Is the failing heart energy depleted? Cardiol Clin 1998;16:633–44,viii.
64. Kennedy JA, Horowitz JD. Effect of trimetazidine on carnitine palmitoyltransferase-1 in the rat heart. Cardiovasc Drugs Ther 1998;12:359–63.
65. Kennedy JA, Kiosoglous AJ, Murphy GA, Pelle MA, Horowitz JD. Effect of perhexiline and oxfenicine on myocardial function and metabolism during low-flow ischemia/reperfusion in the isolated rat heart. J Cardiovasc Pharmacol 2000;36:794–801.
66. Kennedy JA, Unger SA, Horowitz JD. Inhibition of carnitine palmitoyltransferase-1 in rat heart and liver by perhexiline and amiodarone. Biochem Pharmacol 1996;52:273–80.
67. Kirimoto T, Nobori K, Asaka N, Muranaka Y, Tajima K, Miyake H. Beneficial effect of MET-88, a gamma-butyrobetaine hydroxylase inhibitor, on energy metabolism in ischemic dog hearts. Arch Int Pharmacodyn Ther 1996;331:163–78.
68. Kissling G. Oxygen consumption and substrate uptake of the hypertrophied rat heart in situ. Basic Res Cardiol 1980;75: 185–92.
69. Kissling G, Blickle B, Pascht U. Modified heart-lung preparation for the evaluation of systolic and diastolic coronary flow in rats. Am J Physiol Heart Circ Physiol 2000;278:H277–84.
70. Kissling G, Rupp H. The influence of myosin isoenzyme pattern on increase in myocardial oxygen consumption induced by catecholamines. Basic Res Cardiol 1986;81:Suppl 1:103–15.
71. Kjekshus JK, Simonsen S, Bohmer T. Effect of carbocromen induced coronary vasodilatation on myocardial metabolism in coronary artery disease. Clin Cardiol 1978;1:74–9.
72. Klaus W, Guttler K. Influence of carbocromen on some metabolic parameters of isolated working guinea pig hearts. Adv Myocardiol 1982;3:571–5.
73. Kong JY, Rabkin SW. Palmitate-induced cardiac apoptosis is mediated through CPT-1 but not influenced by glucose and insulin. Am J Physiol 2002;282:H717–25.
74. Korb H, Hoeft A, Hunneman DH, et al. Changes in myocardial substrate utilisation and protection of ischemic stressed myocardium by oxfenicine [(S)-4-hydroxyphenylglycine]. Naunyn Schmiedebergs Arch Pharmacol 1984;327:70–4.
75. Leong HS, Grist M, Parsons H, et al. Accelerated rates of glycolysis in the hypertrophied heart: are they a methodological artifact? Am J Physiol 2002;282:E1039–45.
76. Lewis JF, DaCosta M, Wargowich T, Stacpoole P. Effects of dichloroacetate in patients with congestive heart failure. Clin Cardiol 1998;21:888–92.
77. Liang X, Schulz H. 4-bromotiglic acid, a novel inhibitor of thiolases and a tool for assessing the cooperation between the membrane-bound and soluble beta-oxidation systems of rat liver mitochondria. Biochemistry 1998;37:15548–54.
78. Litwin SE, Raya TE, Anderson PG, Litwin CM, Bressler R, Goldman S. Induction of myocardial hypertrophy after coronary ligation in rats decreases ventricular dilatation and improves systolic function. Circulation 1991;84:1819–27.
79. Lopaschuk GD, Wall SR, Olley PM, Davies NJ. Etomoxir, a carnitine palmitoyltransferase I inhibitor, protects hearts from fatty acid-induced ischemic injury independent of changes in long chain acylcarnitine. Circ Res 1988;63:1036–43.
80. Lu C, Dabrowski P, Fragasso G, Chierchia SL. Effects of trimetazidine on ischemic left ventricular dysfunction in patients with coronary artery disease. Am J Cardiol 1998;82:898–901.
81. Maddaiah VT, Miller PS. Effects of ammonium chloride, salicylate, and carnitine on palmitic acid oxidation in rat liver slices. Pediatr Res 1989;25:119–23.
82. Manchanda SC, Krishnaswami S. Combination treatment with trimetazidine and diltiazem in stable angina pectoris. Heart 1997;78:353–7.
83. Mascaro C, Acosta E, Ortiz JA, Marrero PF, Hegardt FG, Haro D. Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. J Biol Chem 1998;273:8560–3.
84. McCormack JG, Barr RL, Wolff AA, Lopaschuk GD. Ranolazine stimulates glucose oxidation in normoxic, ischemic, and reperfused ischemic rat hearts. Circulation 1996;93:135–42.
85. McCormack JG, Stanley WC, Wolff AA. Ranolazine: a novel metabolic modulator for the treatment of angina. Gen Pharmacol 1998;30:639–45.
86. Miyata S, Minobe W, Bristow MR, Leinwand LA. Myosin heavy chain isoform expression in the failing and nonfailing human heart. Circ Res 2000;86:386–90.
87. Montessuit C, Papageorgiou I, Tardy I, Lerch R. Effect of nutritional state on substrate metabolism and contractile function in postischemic rat myocardium. Am J Physiol 1996;271:H2060–70.
88. Nakano M, Kirimoto T, Asaka N, et al. Beneficial effects of MET-88 on left ventricular dysfunction and hypertrophy with volume overload in rats. Fundam Clin Pharmacol 1999;13:521–6.

89. Neubauer S, Hu K, Horn M, et al. Functional and energetic consequences of chronic myocardial creatine depletion by beta-guanidinopropionate in perfused hearts and in intact rats. J Mol Cell Cardiol 1999;31:1845–55.
90. Obermaier-Kusser B, Muhlbacher C, Mushack J, et al. Further evidence for a two-step model of glucose-transport regulation. Inositol phosphate-oligosaccharides regulate glucose-carrier activity. Biochem J 1989;261:699–705.
91. Ojamaa K, Samarel AM, Klein I. Identification of a contractile-responsive element in the cardiac alpha-myosin heavy chain gene. J Biol Chem 1995;270:31276–81.
92. Ol'binskaia LI, Golokolenova GM. Use of mindronate in cardiac insufficiency in patients with ischemic heart disease. Klin Med (Mosk) 1990;68:39–42.
93. Olowe Y, Schulz H. Regulation of thiolases from pig heart. Control of fatty acid oxidation in heart. Eur J Biochem 1980;109:425–9.
94. Olowe Y, Schulz H. 4-Bromocrotonic acid, an effective inhibitor of fatty acid oxidation and ketone body degradation in rat heart mitochondria. On the rate-determining step of beta-oxidation and ketone body degradation in heart. J Biol Chem 1982;257:5408–13.
95. Osterloh J, Cunningham W, Dixon A, Combest D. Biochemical relationships between Reye's and Reye's-like metabolic and toxicological syndromes. Med Toxicol Adverse Drug Exp 1989;4:272–94.
96. Panchal AR, Stanley WC, Kerner J, Sabbah HN. Beta-receptor blockade decreases carnitine palmitoyl transferase I activity in dogs with heart failure. J Card Fail 1998;4:121–6.
97. Pepine CJ, Wolff AA. A controlled trial with a novel anti-ischemic agent, ranolazine, in chronic stable angina pectoris that is responsive to conventional antianginal agents. Ranolazine Study Group. Am J Cardiol 1999;84:46–50.
98. Portilla D, Dai G, Peters JM, Gonzalez FJ, Crew MD, Proia AD. Etomoxir-induced PPARalpha-modulated enzymes protect during acute renal failure. Am J Physiol Renal Physiol 2000;278:F667–75.
99. Qi M, Shannon TR, Euler DE, Bers DM, Samarel AM. Downregulation of sarcoplasmic reticulum Ca(2+)-ATPase during progression of left ventricular hypertrophy. Am J Physiol 1997;272:H2416–24.
100. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev 1998;14:263–83.
101. Ratunova TM, Bauman VR, Kalvin'sh II. The cardioprotective action of carnitine and its structural analog 3-(2,2,2-trimethylhydrazine)propionate on cardiac energy metabolism in experimental occlusion of the coronary artery in rats. Farmakol Toksikol 1989;52:24–7.
102. Remondino A, Rosenblatt-Velin N, Montessuit C, et al. Altered expression of proteins of metabolic regulation during remodeling of the left ventricle after myocardial infarction. J Mol Cell Cardiol 2000;32:2025–34.
103. Ribadeau Dumas A, Wisnewsky C, Boheler KR, Ter Keurs H, Fiszman MY, Schwartz K. The sarco(endo)plasmic reticulum Ca(2+)-ATPase gene is regulated at the transcriptional level during compensated left ventricular hypertrophy in the rat. CR Acad Sci III 1997;320:963–9.
104. Rosenblatt-Velin N, Montessuit C, Papageorgiou I, Terrand J, Lerch R. Postinfarction heart failure in rats is associated with upregulation of GLUT-1 and downregulation of genes of fatty acid metabolism. Cardiovasc Res 2001;52:407–16.
105. Rozanski GJ, Xu Z, Zhang K, Patel KP. Altered K+ current of ventricular myocytes in rats with chronic myocardial infarction. Am J Physiol 1998;274:H259–65.
106. Ruderman NB, Saha AK, Vavvas D, Witters LA. Malonyl-CoA, fuel sensing, and insulin resistance. Am J Physiol 1999;276:E1–18.
107. Rupp H. Differential effect of physical exercise routines on ventricular myosin and peripheral catecholamine stores in normotensive and spontaneously hypertensive rats. Circ Res 1989;65:370–7.
108. Rupp H, Benkel M, Maisch B. Control of cardiomyocyte gene expression as drug target. Mol Cell Biochem 2000;212:135–42.
109. Rupp H, Elimban V, Dhalla NS. Modification of subcellular organelles in pressure-overloaded heart by etomoxir, a carnitine palmitoyltransferase I inhibitor. FASEB J 1992;6:2349–53.
110. Rupp H, Elimban V, Dhalla NS. Differential influence of fasting and BM13.907 treatment on growth and phenotype of pressure overloaded rat heart. Mol Cell Biochem 1998;188:209–15.
111. Rupp H, Schulze W, Vetter R. Dietary medium-chain triglycerides can prevent changes in myosin and SR due to CPT-1 inhibition by etomoxir. Am J Physiol 1995;269:R630–40.
112. Rupp H, Wahl R. Influence of thyroid hormones and catecholamines on myosin of swim- exercised rats. J Appl Physiol 1990;68:973–8.
113. Rupp H, Wahl R, Hansen M. Influence of diet and carnitine palmitoyltransferase I inhibition on myosin and sarcoplasmic reticulum. J Appl Physiol 1992;72:352–60.
114. Sack MN, Disch DL, Rockman HA, Kelly DP. A role for Sp and nuclear receptor transcription factors in a cardiac hypertrophic growth program. Proc Natl Acad Sci USA 1997;94:6438–43.
115. Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. Circulation 1996;94:2837–42.
116. Saeed A, McMillin JB, Wolkowicz PE, Brouillette WJ. 3-Amino-5,5-dimethylhexanoic acid. Synthesis, resolution, and effects on carnitine acyltransferases. J Med Chem 1994;37:3247–51.
117. Schmidt-Schweda S, Holubarsch C. First clinical trial with etomoxir in patients with chronic congestive heart failure. Clin Sci (Colch) 2000;99:27–35.
118. Schofield RS, Hill JA. The use of ranolazine in cardiovascular disease. Expert Opin Investig Drugs 2002;11:117–23.
119. Schraven E. Regulation of lipid and carbohydrate metabolism of the heart by carbocromen. Arzneim Forsch 1976;26:197–200.
120. Schraven E, Trottnow D, Fiedler VB. The effect of carbocromen on cardiac cyclic adenosine- monophosphate. Arzneim Forsch 1976;26:200–4.
121. Schulz H. Inhibitors of fatty acid oxidation. Life Sci 1987;40: 1443–9.
122. Schwartz GG, Greyson C, Wisneski JA, Garcia J. Inhibition of fatty acid metabolism alters myocardial high-energy phosphates in vivo. Am J Physiol 1994;267:H224–31.
123. Simkhovich BZ, Meirena DV, Khagi KhB, Kalvin'sh II, Lukevits EI. Effect of a new structural analog of gamma-butyrobetaine-3-(2, 2,2-trimethylhydrazine)propionate (THP) on carnitine level, carnitine-dependent fatty acid oxidation and various indices of energy metabolism in the myocardium. Vopr Med Khim 1986;32: 72–6.
124. Simkhovich BZ, Meirena DV, Khagi KhB, Kalvin'sh II, Lukevits EI. Biochemical characteristics of the anti-ischemic action of the new structural analog of gamma-butyrobetaine 3-(2,2,2,- trimethylhydrazine)propionate. Farmakol Toksikol 1987;50:100–4.
125. Simkhovich BZ, Shutenko ZV, Meirena DV, et al. 3-(2,2,2-Trimethylhydrazinium)propionate (THP)–a novel gamma-butyrobetaine hydroxylase inhibitor with cardioprotective properties. Biochem Pharmacol 1988;37:195–202.
126. Stacpoole PW, Greene YJ. Dichloroacetate. Diabetes Care 1992;15:785–91.
127. Stanley WC. Partial fatty acid oxidation inhibitors for stable angina. Expert Opin Invest Drugs 2002;11:615–29.
128. Stanley WC, Chandler MP. Energy metabolism in the normal and failing heart: potential for therapeutic interventions. Heart Fail Rev 2002;7:115–30.

129. Stanton LW, Garrard LJ, Damm D, et al. Altered patterns of gene expression in response to myocardial infarction. Circ Res 2000;86:939–45.
130. Stephens TW, Higgins AJ, Cook GA, Harris RA. Two mechanisms produce tissue-specific inhibition of fatty acid oxidation by oxfenicine. Biochem J 1985;227:651–60.
131. Taegtmeyer H. Energy metabolism of the heart: from basic concepts to clinical applications. Curr Probl Cardiol 1994;19:59–113.
132. Taegtmeyer H, Overturf ML. Effects of moderate hypertension on cardiac function and metabolism in the rabbit. Hypertension 1988;11:416–26.
133. Takizawa T, Arai M, Yoguchi A, Tomaru K, Kurabayashi M, Nagai R. Transcription of the SERCA2 gene is decreased in pressure-overloaded hearts: A study using in vivo direct gene transfer into living myocardium. J Mol Cell Cardiol 1999;31:2167–74.
134. Tereshchenko SN, Drozdov VN, Levchuk NN, Demidova IV. Plasma hemostasis and biochemical indices in trimetazidine treatment of patients with chronic heart failure. Ter Arkh 1998;70: 41–4.
135. Thadani U, Ezekowitz M, Fenney L, Chiang YK. Double-blind efficacy and safety study of a novel anti-ischemic agent, ranolazine, versus placebo in patients with chronic stable angina pectoris. Ranolazine Study Group. Circulation 1994;90:726–34.
136. Turcani M, Rupp H. Etomoxir improves left ventricular performance of pressure-overloaded rat heart. Circulation 1997;96: 3681–6.
137. van Bilsen M, van der Vusse GJ, Reneman RS. Transcriptional regulation of metabolic processes: implications for cardiac metabolism. Pflugers Arch 1998;437:2–14.
138. van der Lee KA, Vork MM, De Vries JE, et al. Long-chain fatty acid-induced changes in gene expression in neonatal cardiac myocytes. J Lipid Res 2000;41:41–7.
139. van der Vusse GJ, Dubelaar ML, Coumans WA, et al. Metabolic alterations in the chronically denervated dog heart. Cardiovasc Res 1998;37:160–70.
140. van der Vusse GJ, Glatz JF, Stam HC, Reneman RS. Fatty acid homeostasis in the normoxic and ischemic heart. Physiol Rev 1992;72:881–940.
141. Vetter R, Kott M, Rupp H. Differential influences of carnitine palmitoyltransferase-1 inhibition and hyperthyroidism on cardiac growth and sarcoplasmic reticulum phosphorylation. Eur Heart J 1995;16:Suppl C:15–9.
142. Vetter R, Rupp H. CPT-1 inhibition by etomoxir has a chamber-related action on cardiac sarcoplasmic reticulum and isomyosins. Am J Physiol 1994;267:H2091–9.
143. Vik-Mo H, Mjos OD, Neely JR, Maroko PR, Ribeiro LG. Limitation of myocardial infarct size by metabolic interventions that reduce accumulation of fatty acid metabolites in ischemic myocardium. Am Heart J 1986;111:1048–54.
144. Vik-Mo H, Mjos OD, Smiseth O, Riemersma RA, Oliver MF. Acute effects of the coronary vasodilator carbocromen on myocardial oxygen consumption, substrate uptake and mechanical performance in intact dogs. Arzneim Forsch 1981;31:1088–91.
145. Wambolt RB, Lopaschuk GD, Brownsey RW, Allard MF. Dichloroacetate improves postischemic function of hypertrophied rat hearts. J Am Coll Cardiol 2000;36:1378–85.
146. Wayman NS, Hattori Y, McDonald MC, et al. Ligands of the peroxisome proliferator-activated receptors (PPAR-gamma and PPAR-alpha) reduce myocardial infarct size. FASEB J 2002;16:1027–40.
147. Wolf HP, Engel DW. Decrease of fatty acid oxidation, ketogenesis and gluconeogenesis in isolated perfused rat liver by phenylalkyl oxirane carboxylate (B 807-27) due to inhibition of CPT I (EC 2.3.1.21). Eur J Biochem 1985;146:359–63.
148. Wolff AA, Rotmensch HH, Stanley WC, Ferrari R. Metabolic approaches to the treatment of ischemic heart disease: the clinicians' perspective. Heart Fail Rev 2002;7:187–203.
149. Xiao Q, Kenessey A, Ojamaa K. Role of USF1 phosphorylation on cardiac alpha-myosin heavy chain promoter activity. Am J Physiol 2002;283:H213–9.
150. Xiao Q, Ojamaa K. Regulation of cardiac alpha-myosin heavy chain gene transcription by a contractile-responsive E-box binding protein. J Mol Cell Cardiol 1998;30:87–95.
151. Yao KW, Mao LF, Luo MJ, Schulz H. The relationship between mitochondrial activation and toxicity of some substituted carboxylic acids. Chem Biol Interact 1994;90:225–34.
152. Yonekura K, Eto Y, Yokoyama I, et al. Inhibition of carnitine synthesis modulates protein contents of the cardiac sarcoplasmic reticulum Ca^{2+}-ATPase and hexokinase type I in rat hearts with myocardial infarction. Basic Res Cardiol 2000;95:343–8.
153. Young ME, Laws FA, Goodwin GW, Taegtmeyer H. Reactivation of peroxisome proliferator-activated receptor alpha is associated with contractile dysfunction in hypertrophied rat heart. J Biol Chem 2001;276:44390–5.
154. Young ME, Patil S, Ying J, et al. Uncoupling protein 3 transcription is regulated by peroxisome proliferator-activated receptor (alpha) in the adult rodent heart. FASEB J 2001;15:833–45.
155. Zacharowski K, Blackburn B, Thiemermann C. Ranolazine, a partial fatty acid oxidation inhibitor, reduces myocardial infarct size and cardiac troponin T release in the rat. Eur J Pharmacol 2001;418:105–10.
156. Zarain-Herzberg A, Rupp H. Transcriptional modulators targeted at fuel metabolism of hypertrophied heart. Am J Cardiol 1999;83:31H–7H.
157. Zarain-Herzberg A, Rupp H. Therapeutic potential of CPT I inhibitors: cardiac gene transcription as a target. Expert Opin Invest Drugs 2002;11:345–56.
158. Zarain-Herzberg A, Rupp H, Elimban V, Dhalla NS. Modification of sarcoplasmic reticulum gene expression in pressure overload cardiac hypertrophy by etomoxir. FASEB J 1996;10:1303–9.

Correspondence Address
Prof. Heinz Rupp, PhD
Department of Internal Medicine and Cardiology
Molecular Cardiology Laboratory
Karl-von-Frisch-Straße 1
35033 Marburg
Germany
Phone (+49/6421) 286-5032, Fax -8964
e-mail: Rupp@mailer.uni-marburg.de