Therapeutic potential of CPT I inhibitors: Cardiac gene transcription as a target

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Therapeutic potential of CPT I inhibitors: cardiac gene transcription as a target

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Inhibitors of carnitine palmitoyl-transferase I (CPT I), the key enzyme for the transport of long-chain acyl-coenzyme A (acyl-CoA) compounds into mitochondria, have been developed as agents for treating diabetes mellitus type-2. Findings that the CPT I inhibitor, etomoxir, has effects on overloaded heart muscle, which are associated with an improved function, were unexpected and can be attributed to selective changes in the dysregulated gene expression of hypertrophied cardiomyocytes. Also, the first clinical trial with etomoxir in patients with heart failure showed that etomoxir improved the clinical status and several parameters of heart function. In view of the action of etomoxir on gene expression, putative molecular mechanisms involved in an increased expression of SERCA2, the Ca^{2+} pump of sarcoplasmic reticulum (SR) and alpha-myosin heavy chain of failing overloaded heart muscle are described. The first 225 bp of human, rabbit, rat and mouse SERCA2 promoter sequence have high identity. Various *cis*-regularory elements are also given for the promoter of the rat cardiac alpha-myosin heavy chain (MHC) gene. It is hypothesised that etomoxir increases glucose-phosphate intermediates resulting in activation signalling pathway(s) mediated by phosphatases. Regarding the possible direct action of etomoxir on peroxisome proliferator activated receptor alpha (PPAR-α) activation, it could upregulate the expression of various enzymes that participate in beta-oxidation, thereby modulating some effects of CPT 1 inhibition. Any development of alternative drugs requires a better understanding of the signal pathways involved in the altered gene expression. In particular, signals need to be identified which are altered in overloaded hearts and can selectively be re-activated by etomoxir.

Keywords: 2-tetradecylglycidic acid, carnitine palmitoyl-transferase, etomoxir, gene expression, heart failure, myosin, promoter, sarcoplasmic reticulum, SERCA, transcription factor

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1. Introduction

Inhibitors of CPT I, the key enzyme for the transport of long-chain acyl-CoA compounds into mitochondria, have been developed as agents for treating diabetes mellitus type-2 [1,2]. Best characterised are currently 2-tetradecylglycidic acid, clomoxir and it's follow-up compound etomoxir (**Figure 1**). In diabetic animals, etomoxir exhibited a hypoglycaemic and hypolipidaemic action [3,4]. Treatment of Type 2 diabetic patients with etomoxir resulted in an increased carbohydrate oxidation, decrease in fat oxidation and decrease of the glucose appearance rate in the basal state [5]. In contrast to antidiabetic thiazolidine compounds which activate the PPAR gamma [6], CPT I inhibitors have not been examined in large clinical trials. It appears that a major obstacle in the further development of 2-tetradecylglycidic acid

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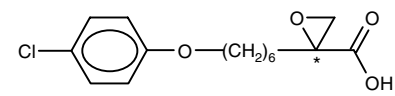
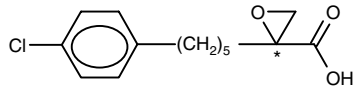
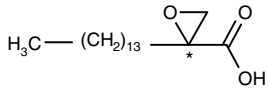


Etomoxir



Clomoxir



2-Tetradecylglycidic acid

Figure 1. Structures of hypoglycaemic CPT I inhibitors with substituted 2-oxiranecarboxylic acids. 2-Tetradecylglycidic acid is also referred to as methyl palmoxirate and clomoxir as POCA. The carbon-2 atom marked by an asterix is asymmetrical and only CoA esters of the R-(+)-enantiomers inhibit CPT I. It should be noted that aliphatic oxirane derivatives are much less chemically reactive than aromatic oxiranes.

or etomoxir relates to their action on cardiac growth. At high doses, cardiac hypertrophy was observed, which has been rated therefore as disadvantageous [7,8]. Since physiological cardiac growth occurs also in the athlete's heart, a judgement simply based on the extent of hypertrophy without taking into account associated changes in cardiomyocyte gene expression appears inadequate. To bypass possible cardiac growth effects of irreversible CPT I inhibitors, the reversible CPT I inhibitor, SDZ-CPI-975, has been developed. SDZ-CPI-975 exhibited a hypoglycaemic action and did neither increase cardiac glucose utilisation nor induce hypertrophy [7,8].

Therefore, it was unexpected that etomoxir has effects on overloaded heart muscle, which are associated with an improved function and can be attributed to selective changes in the dysregulated gene expression of hypertrophied cardiomyocytes [9]. Also, the outcome of the first clinical trial with etomoxir in patients with heart failure might have been unexpected. The study of Schmidt-Schweda and Holubarsch showed that etomoxir improved the clinical status and several parameters of heart function [10]. It was concluded that these preliminary data justify further clinical and mechanistic studies [11]. In view of the novel action of etomoxir on gene expression, the present review focuses on putative molecular mechanisms involved in the expression of SERCA2, the Ca^{2+} pump of SR and MHC of failing overloaded heart muscle.

2. Progression of heart failure and cardiomyocyte gene expression

Despite great research efforts, the prevention of heart failure remains an unmet drug target [12]. With the exception of valvular diseases or coronary artery disease, which can be corrected by surgery or angioplasty, treatment of heart failure remains symptomatic. Current therapeutic regimens often improve heart performance by increasing the oxygen supply to the heart or by unloading the heart. In the failing heart, the neuro-humoral activation involving the sympathetic nervous system and the renin-angiotensin-aldosterone system is an additional target. Current therapy does not, however, take into account that a major problem of diseased hearts arises from a dysregulated gene expression of force-generating cardiomyocytes (**Figure 2**). Since the protein phenotype of the cardiomyocyte is determined by the balance between protein synthesis and degradation, a dysregulated gene expression leads to qualitative or quantitative alterations of the respective proteins.

In view of the potential role of an impaired signal transduction of gene expression of the cardiomyocyte during the early progression of heart failure, studies to identify signals affecting a dysregulated gene expression were initiated. In parallel to the MHC expression [13], the biochemical and functional parameters of SERCA2, which is the major determinant of cellular Ca^{2+} sequestration during diastole [14,15], were examined. Although the activity of the SR Ca^{2+} pump can be modulated acutely by various compounds [16], the possibility of upregulating SERCA2 gene expression using etomoxir became of great interest. In contrast to gene transfer of SERCA2 [14,15], additional proteins appear to be affected in a co-ordinated manner by etomoxir.

3. Diastolic dysfunction and inadequate SERCA2 activity

The importance of the relaxation phase of heart muscle in the pharmacological management of heart failure has long been underestimated [17]. Diastolic dysfunction can be present in mild-to-severe congestive heart failure and is not necessarily coupled to systolic failure. Although diastolic dysfunction can arise from alterations in the visco-elastic properties mainly due to fibrosis, a crucial component involves abnormalities in the cellular systems involved in Ca^{2+} sequestration during diastole. An important consequence of a reduced diastolic relaxation relates to an impaired subendocardial coronary perfusion. The ensuing ischaemia curtails not only the energy-dependent Ca^{2+} uptake of SR, but is expected to have additional detrimental effects on gene expression. A reduced coronary perfusion also contributes to the deleterious geometric remodelling of the heart leading to chamber dilatation.

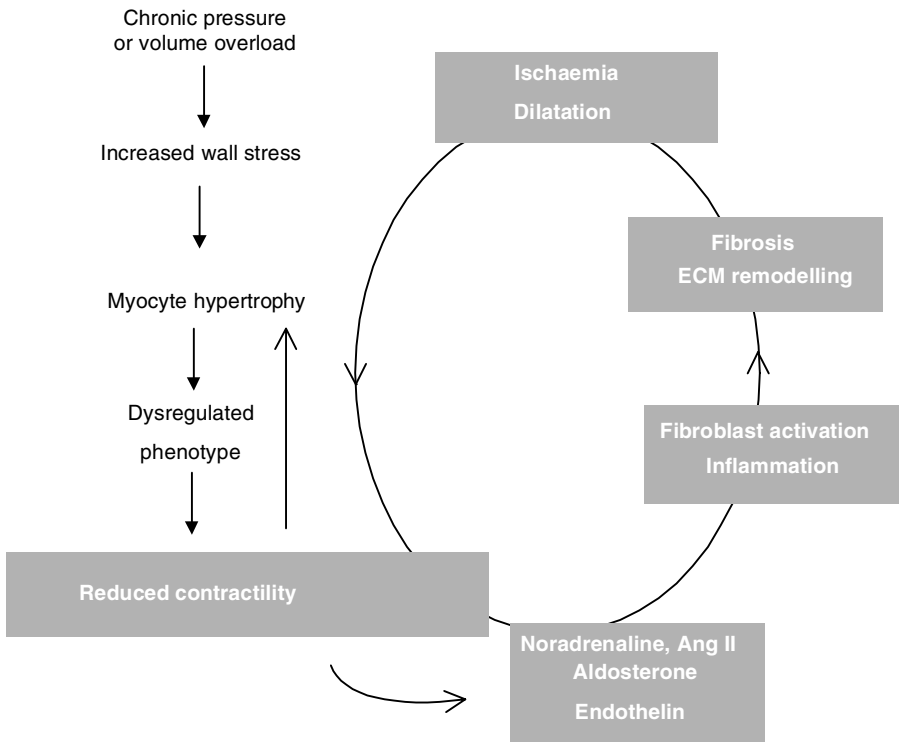


Figure 2. Schematic representation of the progression of structural deterioration of overloaded heart muscle. A major contribution comes from an impaired function of cardiomyocytes arising from a dysregulated gene expression. Currently, well-documented examples involve SERCA2 and myosin heavy chains.

Another aspect of an impaired SR Ca^{2+} sequestration relates to the increased risk of arrhythmogenesis observed in patients with diastolic dysfunction.

4. Etomoxir-induced increase in SERCA2 activity

In patients with failing hearts, the expression of SERCA2 is reduced, which contributes to the observed slowed relaxation and depressed myocardial function [18]. Therefore, drugs which upregulate SERCA2 expression or the associated activity proportionally to the increased size of the hypertrophied cardiomyocyte would be required [19]. Any drug effect will be compared with the effect of thyroid hormones, which are well known to modulate SERCA2 activity. Since thyroid receptors are reduced in pathologically overloaded hearts [20,21], interventions which increase the action of thyroid hormones appear attractive. However, the approach is limited, as thyroid hormones have effects beyond the induction of SERCA2 gene expression. Of crucial relevance is tachycardia and increased oxygen consumption, which have to be avoided in failing hearts. Efforts are made to design thyroid analogues which retain the effect on SERCA2 expression but are free from unfavourable side effects [22,23].

In a series of experiments focusing on metabolic interventions to increase SR function and myosin ATPase activity, it was found that the addition of 0.8% sucrose to the drinking water of rats increased alpha-MHC and SR Ca^{2+}-stimulated ATPase [24]. To further define underlying mechanisms, rats were intermittently fasted and were provided 0.8% sucrose-containing drinking water. The SR Ca^{2+}-stimulated ATPase activity was reduced in rats receiving sucrose-free water, but not in rats receiving sucrose [25]. Since an increased carbohydrate intake can result in an enhanced insulin release probably mediated by incretins [26], the sucrose feeding could partially counteract reduced insulin influences during fasting. Also, in the pressure-overloaded heart, the reduction of the SR Ca^{2+}-stimulated ATPase activity was prevented by the addition of sucrose to drinking water [27].

Further evidence for a role of 'metabolic' signals for SR Ca^{2+} uptake and alpha-MHC expression came from effects of etomoxir, which reduces fatty acid oxidation and in a compensatory manner increases glucose oxidation (**Figure 3**). Treatment with etomoxir increased alpha-MHC and the SR Ca^{2+} uptake rate [28] and the SR Ca^{2+}-stimulated ATPase activity [29]. The increased SR Ca^{2+} uptake rate was attributed to an increased number of SR active Ca^{2+} pumps (E~ P) while phospholamban was not affected [30,31]. The SERCA2a mRNA abundance

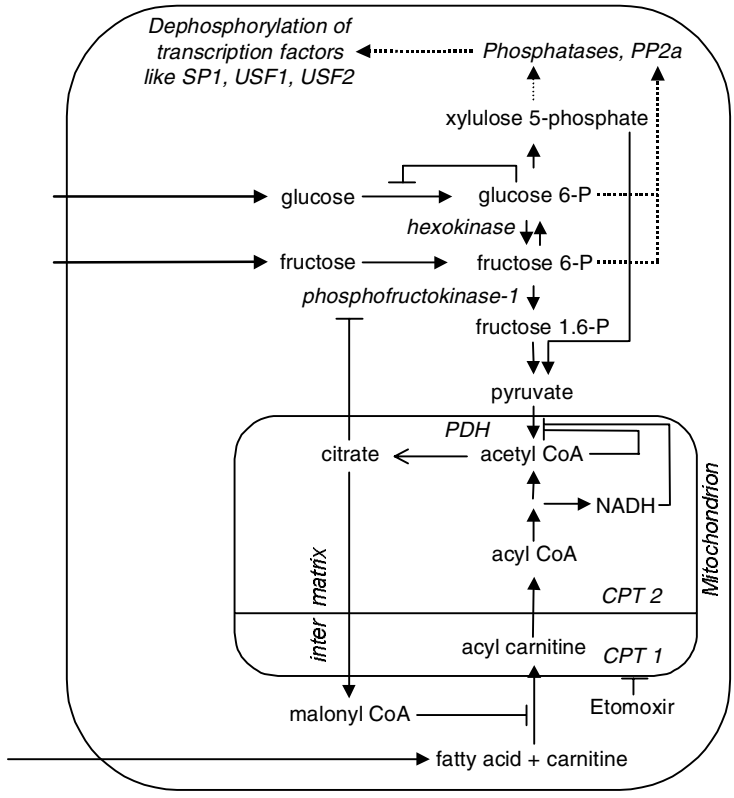


Figure 3. Schematic representation of metabolic pathways affected by a CPT I inhibitor. The uptake of long-chain fatty acids into mitochondria is reduced which results in a compensatory increase in glucose utilisation. Putative phosphorylated sugar metabolites are given, which are candidates for affecting transcription factors, which could be of relevance for SERCA2 and alpha-MHC gene expression. CoA: Coenzyme A; CPT: Carnitine palmitoyl-transferase; NADH: Nicotinamide adenine dinucleotide; PDH: Pyruvate dehydrogenase; PP2a: Phosphatase 2a; SP: Substance P; USF: Upstream stimulatory factor.

was increased after etomoxir treatment in pressure-overloaded hearts while no effect was observed in sham-operated rats [32]. It should be noted that a high dose of etomoxir increased left and right ventricular weight in a proportional manner [28,30,33] while no significant hypertrophy was observed at a lower dose [28,34]. In accordance with a selective influence of etomoxir on the mRNA of pressure-overloaded ventricles [32] is the observation that heart performance was selectively improved in rats with ascending aortic constriction [34]. As both SR function and alpha-MHC expression were increased by etomoxir, it can be deduced that any associated cardiac hypertrophy differs in important parameters from that induced by a pressure overload, which reduces the alpha-MHC proportion.

It should be noted that an etomoxir treatment (80 mg/day) for 3 months did not induce a detectable cardiac growth in patients suffering from heart failure (NYHA functional class II-III; one patient with ischaemic heart disease and nine patients with dilated idiopathic cardiomyopathy) [10]. All patients improved clinically and no patient had to stop taking study medication as a result of side effects. The maximum cardiac output during exercise increased. This was mainly due to an increased stroke volume. During exercise, stroke volume was significantly enhanced for any given heart rate. Also, the left ventricular ejection fraction increased. In acute studies, etomoxir showed neither a positive inotropic effect nor vasodilatory properties. It was hypothesised that the functional improvement arises from an altered gene expression, as observed in animal studies.

5. Metabolic and thyroid influences on SERCA2 activity

Although metabolic influences on gene expression have been examined in detail using integrative physiology approaches, the molecular signals affecting gene transcription remain ill-defined [9]. One could argue that etomoxir affects metabolic signals, which reflect the balance between fatty acid oxidation and glycolytic flux. Since etomoxir increases glucose uptake

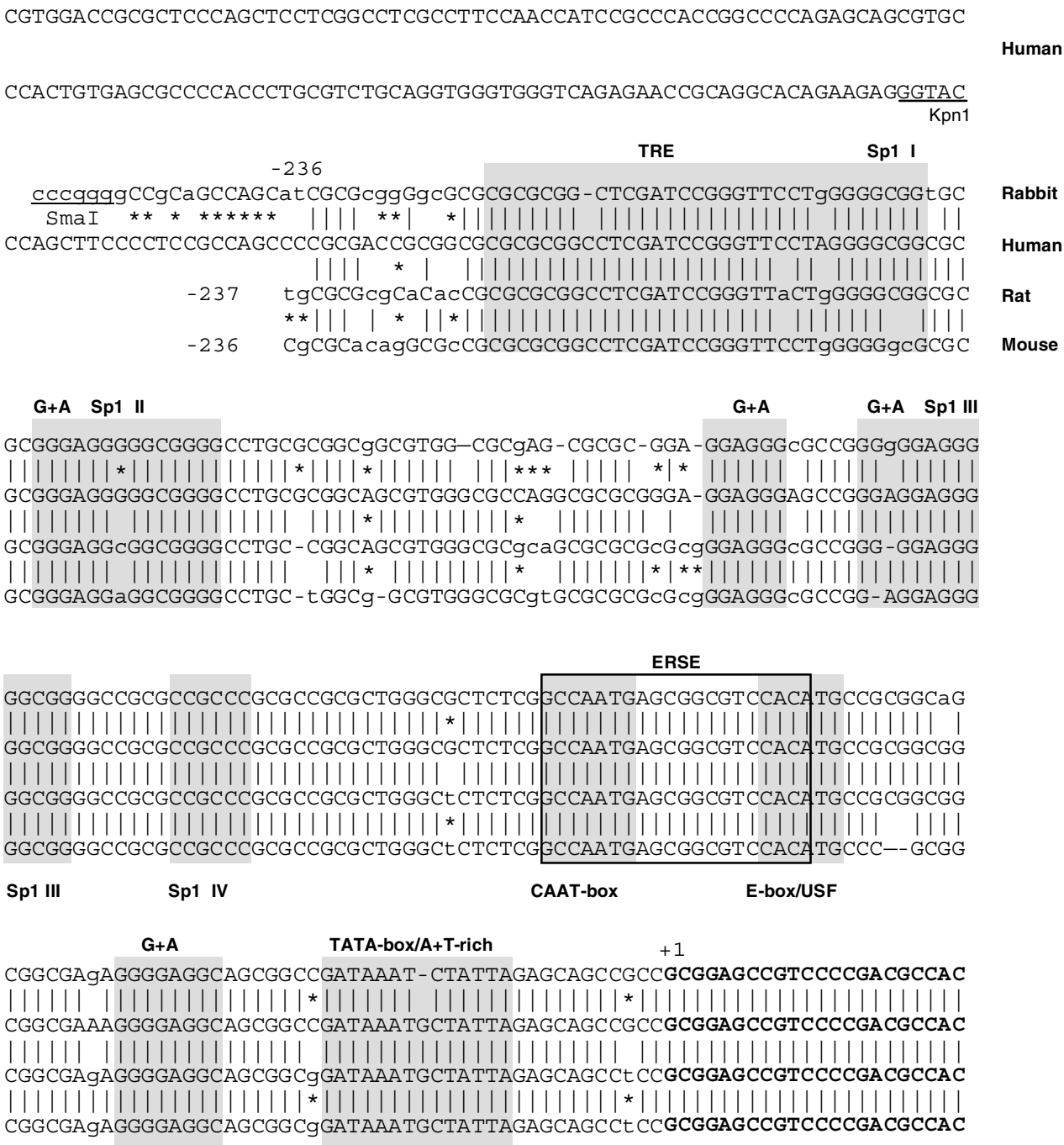


Figure 4. Comparison of human, rabbit, rat and mouse proximal SERCA2 promoter sequences. The identity in nucleotide sequence between different species is indicated by a vertical line (|). Homology only between two sequences is indicated by an asterisk (*). The consensus sequences for various regulatory elements are in gray areas. The 5'-non-translated sequence of the first exonis in bold letters. MacVector 6.5.3, Blast NCBI and Genomatix MatInspector professional 5.1 were used to align sequences and to identify putative binding motifs.

and utilisation, it consequently cannot be stated whether putative signals arise from a reduced or increased concentration of intermediate metabolites (**Figure 3**). It is noteworthy in this respect that the compound BM13.907, which increases glucose transporter translocation, did neither increase alpha-MHC expression nor SR Ca^{2+} ATPase activity [35]. In contrast to etomoxir, BM13.907 reduced the alpha-MHC proportion in parallel with the induced cardiac hypertrophy. When considering potential metabolic signals, one has also to take into account that etomoxir exhibits properties of a PPAR-α activa-

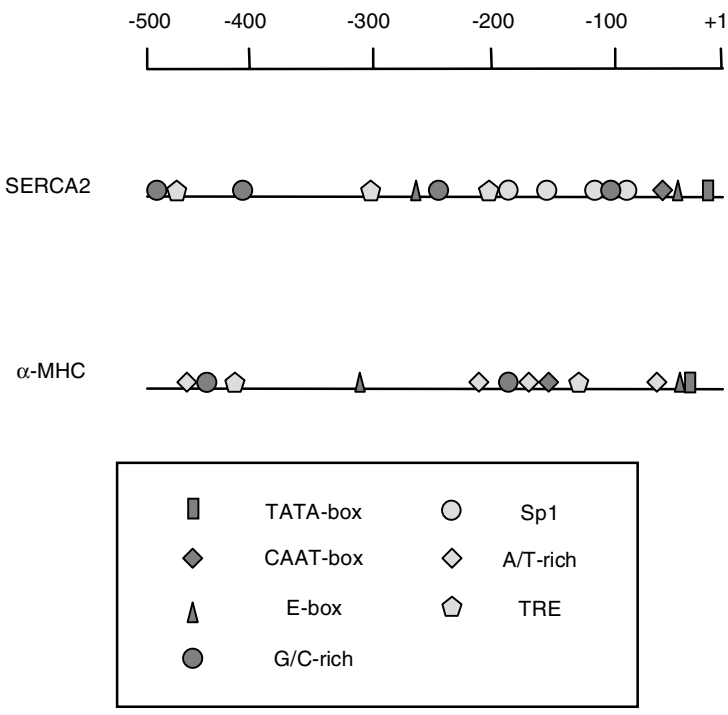


Figure 5. Schematic representation of the various cis-regularory elements found in the promoters of the rat SERCA2 and cardiac alpha-MHC genes. MacVector 6.5.3 and Genomatix MatInspector professional 5.1 were used to identify putative binding motifs.

tor [36]. Since the PPAR-α expression is deactivated in overloaded hypertrophied hearts [37], this possible action of etomoxir should not be dismissed.

In view of the marked effects of thyroid hormones on SERCA2 and alpha-MHC, the question arises whether etomoxir recruits thyroid-linked signals. In a comparative study on SR Ca^{2+} transport, the effects of triiodothyronine and etomoxir were compared [33]. Etomoxir increased cardiac chamber growth evenly, but hyperthyroidism resulted in an over-proportionally higher right ventricular weight. Both interventions increased the proportion of alpha-MHC. The rate of SR Ca^{2+} uptake was increased to a greater extent in hyperthyroid rats than in etomoxir-treated rats. Levels of the SR Ca^{2+} pump modulator phospholamban were moderately decreased in hyperthyroid rats but not in etomoxir-treated rats. The *in vitro* incorporation of ^{32}P into phospholamban, catalysed by protein kinase A, was greatly reduced in hyperthyroid rats, indicating an increased *in vivo* phosphorylation. Etomoxir did not affect phospholamban phosphorylation. Thus, both a higher *in vivo* phospholamban phosphorylation state and a greater number of active SR Ca^{2+} pumps contributed to an increased rate of SR Ca^{2+} uptake in hyperthyroidism. The etomoxir treatment primarily increased the number of active SR Ca^{2+} pumps. These data would not support the contention that the effects induced by etomoxir arise directly from thyroid signalling. In view of the complex interactions between thyroid signalling [38] and metabolism [39], effects at the level of thyroid receptors could, however, be possible.

6. SERCA2 gene 5′-regulatory region as metabolic target

Seven different SERCA isoforms have been cloned that are encoded by three distinct SERCA genes named SERCA1, SERCA2 and SERCA3. The SERCA2 gene encodes two isoforms, SERCA2a and SERCA2b, produced by alternative splicing, which are expressed in a tissue specific manner [40]. The SERCA2 gene is expressed at a 10- to 50-fold higher level in cardiac and slow-twitch skeletal muscle compared with other tissues, with the main isoform being SERCA2a. The 5′-regulatory region of the mouse, rat, rabbit and human SERCA2 genes have been cloned and sequenced [41-44]. The sequence of the proximal promoter region (- 225 bp) of the SERCA2 genes is highly conserved and contains a TATA-like-box (5′-GATAAA-3′), an E-box/USF consensus sequence (5′-CACATG-3′), a CAAT-box (5′-GCCAAT-3′), four consensus Sp1 binding sites (5'-GGGCGG-3' and 5′-CCGCCC-3'), four 5'-GGGAGG-3' sequences that can also bind Sp1 and possibly other transcription factors and a thyroid hormone responsive element (TRE) (5'-GGCCTC-GATCCGGGTTC/ACTG/A-3') (**Figure 4**). The entire

functional 5'-regulatory region of the SERCA2 gene is most likely confined within the 1.5 kb upstream from the transcription initiation site. Upstream from this region there is no homology among the SERCA2 gene of the four species cloned. In addition, in the human gene, several families of repeated sequences (L2, Alu, CAAAA, MER, MIR, LIMB etc.) are located upstream from position -1.7 kb and span, in a continuous manner, > 40 kb. Although, the proximal promoter region is necessary and sufficient for muscle specific expression, other distal sequences contained within 1.5 kb of regulatory region are probably important for regulated expression of the gene during cardiac hypertrophy. Among the DNA *cis*-elements present in the distal promoter region are potential binding sites for: GATA-4, -5, -6, Nkx-2.5/Csx, octamer factor-1 (OTF1), upstream stimulatory factor (USF), myogenic enhancer factor-2 (MEF2), serum response factor (SRF), PPAR/RXR, activator protein-2 (AP2) and thyroid hormone receptors (TR), as well as E-box and M-CAT sequences.

Functional studies have shown that the proximal promoter region of the SERCA2 gene is sufficient to regulate the expression of the SERCA2 gene during myogenic differentiation of the mouse skeletal cell lines C_2C_{12} and Sol8 [41,45]. This region also confers high transcriptional activity in cardiomyocytes. In Sol8 cells, an E-box/A+T-rich element located in the distal 5′-regulatory region of the rabbit SERCA2 gene has been suggested to contribute to the muscle-specific expression of this gene [46]. It has been reported that in doxorubicin (DXR)-induced cardiomyopathy, SR Ca^{2+} transport is decreased, as is the SERCA2 mRNA and gene transcriptional activity [47]. This effect was prevented by a specific inhibitor of p44/42 MAPK. ANF, beta-MHC (but not alpha-MHC), Egr-1 and TAFII250 expression were increased by DXR treatment [48]. Egr-1 is a nuclear phosphoprotein with three zinc fingers that bind to the GC-rich element (CGCCCCCGC) and can modulate transcription through repressive and activating domains, which involves also competition with Sp1 [49,50]. This GC-rich sequence is present in the Egr-1 gene, the proximal promoter of the SERCA2 and alpha-MHC genes (**Figure 5**). Transfection assays located the DXR responsive element within the proximal SERCA2 promoter region and overexpression of Egr-1 decreased the transcriptional activity of the SERCA2 gene [47]. These findings suggested that reactive oxygen species mediate their transcriptional effect on the SERCA2 gene *via* p44/42 MAPK and Egr-1. To date, other transcriptional mechanisms influencing the SERCA2 gene in pathological conditions are incompletely understood.

Recently, it has been shown that the Ca^{2+}-dependent phosphatase calcineurin plays a very important role in the development of pressure overload cardiac hypertrophy through the activation of NF-AT3 [51,52]. A potential target site for treatment of cardiac hypertrophy is thus the inhibition of calcineurin activity. The inhibitor cyclosporin A and FK506 prevented cardiac hypertrophy with the subsequent reversion of the hypertrophied heart phenotype [51,52]. The mRNA levels for SERCA2, skeletal alpha-actin and ANF were normalised. However, as a result of the side effects of the currently known calcineurin inhibitors, it is at present not a useful therapeutic approach.

It has been shown that the rat and rabbit SERCA2 promoter/reporter fusion gene constructs with the proximal promoter sequence can be trans-activated by the thyroid hormone receptor (TR) isoforms alpha1 and beta1 and that this region can bind both receptors *in vitro* [53-55]. It was recently reported that the thyroid hormone receptor isoforms TR-alpha1, TR-alpha2 and TR-beta1 undergo different changes in the pattern of expression in physiological *versus* pathological cardiac hypertrophy [21]. The three TR subtypes were downregulated in pressure overload animal models and were treated with phenylephrinein in cultured cardiomyocytes. In contrast, TR-beta1 was upregulated in cardiomyocytes culture-treated with triiodothyronine or in exercise-induced cardiac hypertrophy [21]. These findings explain, at least in part, why a decreased SERCA2 expression is observed in certain cardiac hypertrophy models, in spite of normal circulating levels of thyroid hormones. Using the rat heart-derived cell line H9c2, it has been demonstrated that the transcription factor, MEF2a, increases the transcription of the rat SERCA2 gene induced by TR-alpha1 and TR-beta1 [55].

Three TREs have been described in the rat SERCA2 gene. TRE-1 and TRE-2 are located upstream from the proximal promoter, whereas TRE-3 is located within the first 250 bp of the promoter sequence. Similar sequences are also present in the rat, rabbit and human genes. TRE-1 is a direct repeat of two half sites separated by four nucleotides that can be contacted preferably by the monomeric form of TR-apha1, homodimers of TR-beta or by heterodimers TR-alpha/RXR. In addition, retinoic acid can also stimulate the SERCA2 gene expression by a mechanism which did not involve the complete TRE-1, but only the 5′-half site. In contrast, TRE-2 and TRE-3 are inverted palindromes of two half sites separated by 4 or 6 nucleotides, which can bind homodimers of TR-alpha1 or TR-beta [54].

Many research groups have documented that in both animal models of cardiac hypertrophy and in patients with heart failure, the mRNA expression of SERCA2 is decreased [56]. Recently, it has been demonstrated that the decreased SERCA2 mRNA levels arise from a reduced gene transcription [57-60]. The Sp1-factor mRNA levels as well the Sp1-factor binding activity has been shown to be increased in pressure-overloaded hearts [50,61]. Sp1 has been reported to be necessary for induction of the skeletal alpha-actin in pressure overload cardiac hypertrophy. Recently, it was reported that two proximal Sp1 binding sites within the SERCA2 promoter (Sp1 I and Sp1 III) (**Figure 4**) are responsible for the Sp1 mediated transcriptional inhibition observed in pressure overload cardiac hypertrophy [61]. Recent evidence also suggests that activation of the p38-MAPK pathway may participate in the downregulation of SERCA2 gene transcription in response to a hypertrophic growth of the heart [62]. It can,

therefore, be suggested that a decreased SERCA2 expression leads to a dysregulation of cardiac myoplasmic free Ca^{2+} and ultimately to activation of NF-AT pathway of cardiac hypertrophy. NF-AT3, when dephosphorylated, is able to interact with GATA-4 bound to its consensus sequence, thereby affecting the transcriptional activity of target genes. Thus, there is a possibility that GATA binding sites in the SERCA2 promoter are involved in downregulating SERCA2 expression during cardiac hypertrophy.

In pressure overload cardiac hypertrophy, the heart shifts from a normal preference for fatty acid oxidation to a predominant glucose oxidation [37,63]. This shift in substrate utilisation is accompanied by deactivation of PPAR-α [63,64]. PPAR-α is a well-known factor that activates the transcription of genes encoding enzymes involved in fatty acid oxidation. The decrease in PPAR-α expression during the development of cardiac hypertrophy could contribute to the pathological remodelling associated with contractile dysfunction of the heart. A recent report suggests that p38 activated MAPK activates PPAR-α as a mechanism of the energy metabolic stress response in cardiac hypertrophy [64]. The SERCA2 gene promoter contains two consensus sequences (5'-(A/G)GG(T/G)NA-3') that may bind class II members of the nuclear receptor super family, which may function as fatty acid response elements. However, further experimental data are required.

7. Regulation of alpha-MHC and SERCA2 gene expression by glucose

Regarding the effect of glucose on SERCA2 expression, it has been demonstrated in cultured vascular smooth muscle cells that glucose and 2-deoxyglucose increase the level of SERCA2 mRNA expression. The latter is also stimulated by insulin [65]. In contrast, 3-O-methylglucose, which can enter the cell but cannot be phosphorylated, had no effect. Recent results (from our laboratory) demonstrate that cardiomyocytes cultured in the presence of high glucose (25 mM) exhibit an increased level of SERCA2 mRNA with respect to cells cultured in low (5 mM) glucose. An increased transcriptional activity of the rabbit and human SERCA2 promoters (unpublished) were also observed. Interestingly, 2-deoxyglucose in the presence of low glucose and high glucose also increased both the SERCA2 mRNA and transcriptional activity (unpublished). This effect could be due to the fact that 2-deoxyglucose enters the cardiomyocyte and can be phosphorylated to 2-deoxyglucose-6-phosphate. Since 2-deoxyglucose cannot be further metabolised, it accumulates, mimicking the effect of high glucose. It can thus be hypothesised that sugar metabolites, such as glucose-6-P, fructose-6-P and xylulose-5-P, could activate phosphatases like PP2a, which in turn can dephosphorylate transcription factors like USF1. Glucose or fructose appear, therefore, to play a crucial role in the regulation of gene expression of alpha-MHC and SERCA2 through a complex interaction of transcription factors that may include USF1.

When cultured cardiomyocytes were treated with long-chain fatty acids (palmitic and oleic acids) with or without glucose, the level of enzymes involved in the oxidation of fatty acids was increased. However, the mRNA abundance of proteins involved in the uptake and metabolism of glucose was not altered. Similarly, ANF and SERCA2 mRNA levels were not changed [66]. It is interesting to note that recent studies suggest that etomoxir and other CPT-1 inhibitors potentially can act as ligands for PPAR-α [36]. Taken together, the functional relevance of these sites on SERCA2 expression by PPAR-α is not clear and needs to be examined further.

The alpha-MHC gene proximal promoter contains several well-identified regulatory elements [13]. Among them is a strong TRE, two A/T-rich regions which can bind MEF-2, three CArG-boxes and an E-box/USF motif (CACGTG) close to the TATA-box (**Figure 5**). The E-box/USF motif seems to be necessary for high levels of basal transcription and in response to a contractile stimulus [67,68]. This site can bind the basic helix-loop-helix leucine zipper (bHLHZ) transcription factor USF1. Overexpression of USF1 increased the transcription of the cardiac alpha-MHC gene [68]. In support of the above observation have been studies in thyroidectomised rats by the groups of Morkin and Dillmann which demonstrated that glucose or fructose induced the expression of alpha-MHC from an undetectable level to about 20 - 30% of total myosin, suggesting that the regulation of cardiac MHC genes may involve a primary signal related to dietary carbohydrates [69,70].

It is important to mention that among the proteins induced in response to stress in the endoplasmic reticulum (ER) are the glucose-regulated proteins (GRP), GRP78 and GRP94. The induction of ER stress proteins is part of the cellular response to the accumulation of unfolded proteins in the ER. In mammalian cells, the ER stress responsive element (ERSE) is a sequence that is necessary for the transcriptional induction of ER chaperones in response to ER stress. The ERSE consensus sequence is CCAATN9CCACG. The CCAAT part of this element is known to bind the general transcription factor NF-Y/CBF, whereas the CCACG confers specificity on the unfolded protein response (UPR). It has been extensively documented that the basic leucine zipper (bZIP) protein ATF6, is a protein that binds to the CCACG part of the ERSE. GRP-78 requires ATF6 for induction through ERSE [71]. It is well known that the expression of GRP-78 is induced as a result of glucose deprivation. GRPs are, however, constitutively synthesised in the absence of stress. Glucose starvation and 2-deoxyglucose can induce GRP-78 gene expression. It has recently been reported that GRP-78 is increased in the embryonic mouse heart and is also induced following hypoglycaemic stress [72].

The SERCA2 expression can also be induced by ER stress agents, such as intracellular Ca^{2+} depletion or by agents that affect glycosylation of proteins like tunicamycin, dithiothreitol or L-azetidine-2-carboxylic acid [73,74]. Recently, Dillmann and co-workers have reported that the proximal SERCA2 pro-

moter region contains a functional consensus ERSE that is active in response to Ca^{2+} depletion [75]. The ERSE present in the SERCA2 promoter (CCAATN9CCACA) could bind NF-Y/CBF, YY-1 and ERSF (**Figure 4**). In that study, ATF6 transactivates the expression of the rat SERCA2 gene and it has been proposed that ATF6 may interact with NF-Y and also make contact with the sequence CCACA sequence of the ERSE, although this sequence differs one bp from the consensus sequence CCACG. The SERCA2 gene also has a proximal E-box/USF element between the CAAT-box and the TATA-box and is similar to the E-box found in the alpha-MHC gene. This element forms part of the ERSE and may play an important role in the basal and regulated expression of this gene. It is interesting to speculate that ATF6 may not bind directly with that sequence, but rather interact with another factor(s) that actually binds to the E-box/USF sequence (CACATG), which is part of the ERSE. However, it is not known if the ERSE present in the SERCA2 gene responds to glucose deprivation, increasing or decreasing the transcription of the gene.

8. Expert opinion

In animal experiments, the CPT I inhibitor and PPAR-α activator etomoxir, has been shown to improve the function of overloaded hearts. In contrast to established drugs for the failing heart, etomoxir has no acute effects but involves an altered expression of dysregulated genes which are important for myocardial function. Depending on the half-life of affected proteins, a few days up to several weeks would be required for observing any effect. The findings of the animal studies are supported by the pilot clinical trial of Schmidt-Schweda and Holubarsch [10]. A multi-centre Phase II clinical trial named ERGO-1 (Medigene AG, München) is currently underway in patients with heart failure to examine the clinical efficacy and the optimal dosage of etomoxir.

Since etomoxir increases glucose utilisation, it could alter sugar metabolites in the cardiomyocyte. In particular, sugar-phosphate intermediates may be affected, resulting in activation signalling pathway(s) mediated by phosphatases. The direct action of etomoxir on PPAR-α activation, could possibly compensate for the decreased levels of PPAR-α in the hypertrophied myocardium. The increase in PPAR-α activity could upregulate the expression of various enzymes that participate also in beta-oxidation, thereby modulating some effects of CPT I inhibition. It appears, however, that a short-term treatment with the pure PPAR-α activator WY-14,643, induces contractile dysfunction (measured *ex vivo*) in pressure-overloaded hearts [76]. It was, therefore, suggested that the switch in fuel utilisation in favour of glucose oxidation of pressure-overloaded hearts may be essential for the maintenance of cardiac output [76].

Any development of alternative drugs requires a better understanding of the signal pathways involved in the etomoxir-induced changes in gene expression. Signals need to be identified which are perturbed in overloaded hearts and can selectively be re-activated by etomoxir. Such studies are also of relevance in view of the increasing evidence that metabolic remodelling can be an important facet of cardiac adaptation to various chronic pathophysiological conditions [77,78]. The elucidation of these signals is expected to provide also answers to the question why cardiac hypertrophy arising from a pathological pressure overload but not from physical exercise, represents a cardiac risk factor.

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