Review

Pharmacological effects of meldonium: Biochemical mechanisms and biomarkers of cardiometabolic activity

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ABSTRACT

Meldonium (mildronate; 3-(2,2,2-trimethylhydrazinium)propionate; THP; MET-88) is a clinically used cardioprotective drug, which mechanism of action is based on the regulation of energy metabolism pathways through L-carnitine lowering effect. L-Carnitine biosynthesis enzyme γ-butyrobetaine hydroxylase and carnitine/organic cation transporter type 2 (OCTN2) are the main known drug targets of meldonium, and through inhibition of these activities meldonium induces adaptive changes in the cellular energy homeostasis. Since L-carnitine is involved in the metabolism of fatty acids, the decline in its levels stimulates glucose metabolism and decreases concentrations of L-carnitine related metabolites, such as long-chain acylcarnitines and trimethylamine-N-oxide. Here, we briefly reviewed the pharmacological effects and mechanisms of meldonium in treatment of heart failure, myocardial infarction, arrhythmia, atherosclerosis and diabetes.

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Contents

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1. Introduction

Meldonium (mildronate; 3-(2,2,2-trimethylhydrazinium) propionate; THP; MET-88) is a clinically used cardioprotective drug, and its mechanism of action includes lowering the L-carnitine content in body tissues. L-Carnitine was discovered more than 100 years ago by the Latvian biochemist Krimbergs, who isolated L-carnitine from meat extract [1]. L-Carnitine is obtained from dietary products, but it can be also biosynthesized from lysine and methionine. The physiological range of L-carnitine concentrations in body tissues is regulated by L-carnitine and organic cation transporters. The roles of L-carnitine in cellular energy metabolism are schematically summarized in Fig. 1. L-Carnitine acts as a substrate of the enzyme carnitine palmitoyltransferase-1 (CPT1), which catalyses the rate-limiting reaction in the transfer of fatty acids (FA) into the mitochondria [2]. The CPT1 activity is allosterically inhibited by malonyl-CoA [3]. In the CPT1-catalysed reaction, L-carnitine and acyl-CoA-activated long-chain FA form long-chain acylcarnitine esters that are shuttled across the mitochondrial membranes by carnitine/acylcarnitine translocase (CACT) [4]. Inside the mitochondrial matrix, the enzyme CPT2 converts the acylcarnitine back

to L-carnitine and long-chain acyl-CoA. Thus, L-carnitine is needed for the translocation of long-chain FA to the mitochondria for β-oxidation and energy production for muscle cells.

In addition, L-carnitine participates in the export of acetyl groups out of the mitochondria [5]. In the mitochondrial matrix, the endoplasmic reticulum lumen and peroxisomes carnitine acetyltransferase (CrAT) catalyses the reversible transfer of acetyl groups between acetyl-CoA and L-carnitine [6] and regulates the cellular pool of CoA [6]. The homeostasis of the acetyl-CoA/CoA ratio regulates glucose metabolism pathways. An increase in this ratio activates pyruvate dehydrogenase kinase (PDK), which phosphorylates and thereby inhibits the pyruvate dehydrogenase complex (PDC), the key rate-limiting step in carbohydrate oxidation [7,8]. It can be concluded that L-carnitine participates in the regulation of both long-chain FA and carbohydrate metabolism and homeostasis of cellular energy metabolism.

2. Biochemical mechanisms of action

Meldonium decreases the availability of L-carnitine and subsequently modulates cellular energy metabolism pathways. The sites of action of meldonium are described below and summarized in Table 1.

2.1. Inhibition of L-carnitine biosynthesis

L-Carnitine biosynthesis in mammals was first characterized almost 40 years ago, and the final step is carried out by an enzyme γ-butyrobetaine hydroxylase (BBOX), which is present in kidney, liver and brain [9,10]. Almost 30 years ago, meldonium was first shown to inhibit rat BBOX in a non-competitive manner and to decrease L-carnitine (by 63.7%) and long-chain acylcarnitine (by 74.3%) levels [11], while later studies revealed that meldonium is a competitive BBOX inhibitor, and the rat BBOX K_m and K_i values for meldonium are 37 μM and 16 μM, respectively [12]. More recently, human BBOX was characterized [13], and similar to the rat enzyme, it was also inhibited by meldonium, though the IC_{50} values (34–62 μM) reported by different groups [13,14] are slightly higher than those IC_{50} values (13–18 μM) observed for the rat enzyme [12,15]. The K_i value of meldonium for human BBOX inhibition was found to be 19 μM [16]. It was also shown that meldonium itself can be metabolized by BBOX, yielding malonic acid semi-aldehyde as a major metabolite [14]. It has also been shown that meldonium does not inhibit other L-carnitine biosynthesis enzymes and does not change the expression of BBOX and other L-carnitine biosynthesis enzymes [17].

2.2. Inhibition of L-carnitine transport

Meldonium-induced L-carnitine concentration-lowering effects were first attributed to only BBOX inhibition, but later studies revealed that meldonium also inhibits L-carnitine transport in the kidneys, with a K_i value of 52.2 μM [18]. Later, it was shown that in kidneys, meldonium acts as an inhibitor of rat organic cation/carnitine transporter type 2 protein (OCTN2), and the K_i value of meldonium for rat OCTN2 was determined to be 41 μM [12]. The reported K_i values for meldonium are lower than the rat plasma concentration of meldonium (68 μM) after 14 days of treatment with a dose of 100 mg/kg [19], indicating that meldonium can effectively increase L-carnitine elimination via urine as observed using ^{13}C-labelled L-carnitine [20]. Interestingly, the K_m value of L-carnitine for OCTN2 in meldonium-treated rats was not significantly increased, though L-carnitine transport in renal brush border membrane vesicles isolated from meldonium-treated rats increased almost 1.9 times, with a V_{max} of 131 pmol/min/mg protein [21]. Meldonium competes with L-carnitine for transport via
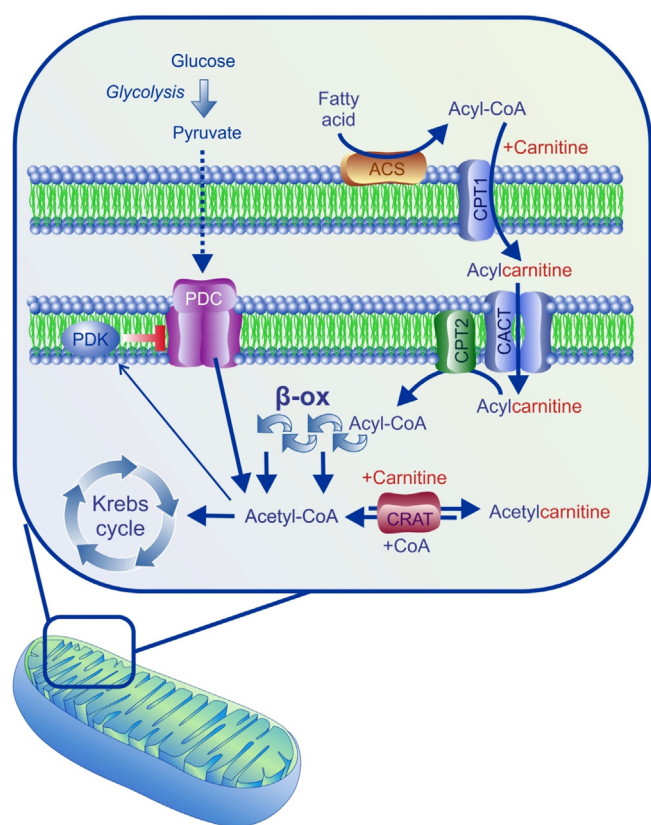

Fig. 1. The role of L-carnitine in fatty acid transport and mitochondrial energy metabolism pathways. Fatty acid (FA) is activated to corresponding acyl-coenzyme A (Acyl-CoA) via long-chain acyl-coenzyme A synthetase (ACS). Then, L-carnitine facilitates acyl-CoA transport into the mitochondria via conversion to respective acylcarnitine by carnitine palmitoyltransferase-1 (CPT1). The resulting acylcarnitine is subsequently transported into the mitochondria by carnitine/acylcarnitine translocase (CACT). In the mitochondrial matrix, acylcarnitine is converted back to acyl-CoA by carnitine palmitoyltransferase-2 (CPT2) and undergoes subsequent β-oxidation (β-ox), producing acetyl-coenzyme A (acetyl-CoA). In glycolysis, glucose is metabolized to pyruvate, which is decarboxylated to acetyl-CoA by the pyruvate dehydrogenase complex (PDC). Acetyl-CoA formed in FA and glucose metabolism processes can be further metabolized in the Krebs cycle or can be converted to acetylcarnitine by carnitine acetyltransferase (CrAT). CrAT catalyses the formation of acetylcarnitine from acetyl-CoA and L-carnitine and thus regulates the acetyl-CoA/free CoA ratio, which is sensed by the pyruvate dehydrogenase kinase (PDK) that inhibits PDC activity.

Table 1
Sites of action of meldonium.

Target	Effect	Characteristics	References
Rat BBOX	Competitive inhibition, unchanged BBOX mRNA expression	Meldonium K_i = 16 μM and K_m = 37 μM,	[11,12,17]
Human recombinant BBOX	Competitive inhibition	Meldonium K_i = 19 μM and IC_{50} = 34–62 μM	[13,14,16]
Rat TMLH	Decreased trimethyllysine excretion via urine	No inhibition, unchanged TMLH mRNA expression	[17]
Rat OCTN2	Competitive inhibition, increase in OCTN2 expression after treatment with meldonium—higher V_{max}	Meldonium K_i = 41 μM, L-Carnitine transport V_{max} = 131 pmol/min/mg protein	[12,21]
Human OCTN2	Inhibition of L-carnitine transport, transporter of meldonium	Meldonium EC_{50} = 21 μM Meldonium uptake K_m = 26 μM	[35,36]
CPT1	No direct inhibition, increase in mRNA expression, decrease in enzyme activity due to decrease in L-carnitine availability	Carnitine-dependent CPT1 activity decreased by 26% in isolated mitochondria from meldonium-treated rats	[19,23,24,37]
CrAT	Weak competitive inhibition	Meldonium K_d = 101 μM, Meldonium IC_{50} values 1.44–20.44 mM (at carnitine concentrations ranging from 62.5 μM to 1000 μM)	[27]
CACT	Weak competitive inhibition, transporter of meldonium (both antiport and uniport)	Meldonium K_i = 530 μM, Meldonium efflux K_m value during antiport is 18 mM	[31]

K_i—half saturation constant; K_m—Michaelis–Menten constant; K_d—dissociation constant; TMLH—trimethyllysine hydroxylase.

OCTN2 [12], and the cardioprotective effect of meldonium depends on its ability to decrease L-carnitine levels in tissue and plasma [19]. Recently it was shown that the inhibition of OCTN2 is a more effective approach for lowering L-carnitine availability and for decreasing the size of the myocardial infarct [22].

2.3. Effects on L-carnitine-dependent enzymes

L-Carnitine is generally thought to be required for the CPT1-dependent formation and transport of long-chain FA carnitine esters into the mitochondrial matrix (Fig. 1). The effects of meldonium treatment on CPT1 activity have been extensively studied (Table 1), and the results largely depend on the L-carnitine concentrations used in the experiment. Meldonium treatment results in increased mRNA levels [23] and protein expression [24] of CPT1. In addition, meldonium has no effect on CPT1 sensitivity to malonyl-CoA inhibition [23]. When a similar amount of external L-carnitine was added to isolated mitochondria from the hearts of meldonium-treated rats, specific CPT1 activity and the L-carnitine-dependent palmitic acid oxidation rate significantly increased [23], but when concentrations of L-carnitine present in the heart tissues of control and meldonium-treated animals were used, the CPT1 activity and the mitochondrial respiration on palmitoyl-CoA decreased by 26% and 27%, respectively [19]; these effects are due to changes in L-carnitine availability because meldonium has no direct inhibitory effect on CPT1. It was also observed that meldonium stimulates

CPT1-independent FA metabolism in mitochondria when palmitoylcarnitine (a product of CPT1 reaction) is used as a substrate [25]. Together, these results indicate that adaptive changes occur in mitochondrial metabolism after meldonium treatment.

CrAT regulates the ratio between acetyl-CoA and free CoA (Fig. 1). Initially, it was thought that meldonium inhibits CrAT and can foster mitochondrial metabolism processes by increasing acetyl-CoA availability [26]. It was later shown that meldonium is a very weak CrAT inhibitor (Table 1) with a K_i value of 5.2 ± 0.6 mM in the presence of L-carnitine in tissues [27], and no inhibition is observed in vivo [19].

In recent years, intestinal metabolism of dietary tertiary amines such as L-carnitine and the subsequent production of trimethylamine-N-oxide (TMAO) has been linked to the development of cardiovascular diseases [28], and the effects of meldonium on intestinal bacteria have also been studied. It was shown that meldonium does not affect bacterial L-carnitine transporters but rather inhibits the production of trimethylamine (a precursor of TMAO that is detrimental to cardiovascular health; Figs. 4 and 5) by intestinal microbiota in a non-antibacterial manner, which is most likely by inhibiting carnitine oxygenase [20].

2.4. Effects on cellular and mitochondrial transporters

CACT mediates acylcarnitine transport through the inner mitochondrial membrane to the mitochondrial matrix in the exchange
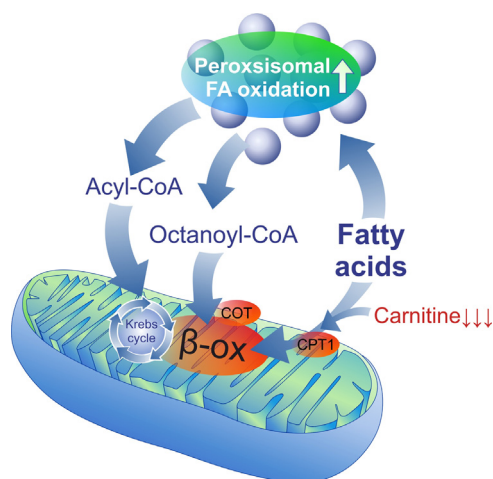
Fig. 2. Meldonium treatment-induced redirection of long-chain FA metabolism from mitochondria to peroxisomes. The meldonium-induced decrease in the L-carnitine concentration reduces the carnitine palmitoyltransferase-1 (CPT1)-dependent transport of long-chain fatty acids and stimulates β-oxidation (β-ox) in mitochondria. In turn, peroxisomal fatty acid (FA) oxidation is upregulated. The redirection of FA metabolism from mitochondria to peroxisomes and the stimulation of mitochondrial β-ox protect mitochondria against FA metabolite overload. COT—carnitine O-octanoyltransferase.

Fig. 3. The role of acylcarnitines in the regulation of glucose metabolism. Acylcarnitines decrease glucose utilization via the inhibition of insulin signalling-related glucose uptake via glucose transporter type 4 (GLUT4) and reduction of pyruvate metabolism by blocking the pyruvate dehydrogenase complex (PDC).

of L-carnitine [29,30] (Fig. 1). In 2008, Oppedisano et al. demonstrated that meldonium directly inhibits CACT [31]. Meldonium binds to CACT at the same site as L-carnitine and acts as a competitive inhibitor. However, the K_i of meldonium is 530 μM, indicating weak inhibitory potency (Table 1). In addition, it has been demonstrated that meldonium can be transported inside the mitochondria by CACT, thus allowing direct action of meldonium on intramitochondrial enzymes [31].

Sarcoplasmic reticulum Ca²⁺-ATPase (SERCA) ensures calcium uptake in the sarcoplasmic reticulum, and meldonium has been shown to prevent an ischemia-induced decrease in SERCA protein expression and a decrease in Ca²⁺ uptake V_{max} [32–34].

2.5. Regulation of energy metabolism

Initially, it was suggested that a meldonium-induced decrease in L-carnitine content inhibits FA oxidation in the heart [11,38], which is known to be beneficial in treating cardiac disorders [39]. However, further studies have shown that meldonium treatment does not induce any changes in the total cardiac FA oxidation [23,25]. It has been demonstrated that meldonium treatment results in significantly decreased CPT1 activity and CPT1-dependent β-oxidation in cardiac mitochondria [19]. Further study showed stimulated CPT1-independent β-oxidation after meldonium treatment (Fig. 2) [25]. In addition, it has been shown that the meldonium-induced long-term inhibition of L-carnitine-dependent mitochondrial FA oxidation is compensated by the increase in peroxisomal FA metabolism, resulting in an unchanged overall FA oxidation rate. The compensatory activation of the PPAR-α/PGC1-α signalling pathway was observed [25,40]. The increase in the nuclear content of PPARα and PGC1α resulted in the increased expression of genes related to FA oxidation, the activation of the proliferation of peroxisomes, and the subsequent stimulation of peroxisomal FA oxidation [23,25]. As a result, in peroxisomes, long-chain FA are metabolized to medium- and short-chain acylcarnitines, such as octanoyl- and acetyl-carnitines, which are not toxic to mitochondria and can be easily metabolized in mitochondria without the accumulation of toxic long-chain intermediates (Fig. 2). Thus, the reduction of long-chain FA transport into mitochondria and the simultaneous activation of peroxisomal FA oxidation protect mito-
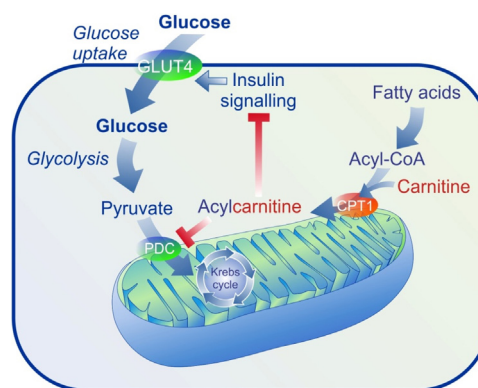
chondria from the accumulation of long-chain acylcarnitines in the ischemic heart [25]. Taken together, the meldonium-induced decrease in L-carnitine availability protects mitochondria against an FA metabolite overload by reducing the formation of long-chain acylcarnitines, stimulating FA mitochondrial utilization and redirecting FA metabolism from mitochondria to peroxisomes.

The effects of the meldonium-induced decreased L-carnitine availability on glucose metabolism regulation have also been studied extensively. It has been shown that a 20-day meldonium treatment induces increases in gene and protein expression related to glucose metabolism [24]. The expression increases in GLUT4 and insulin receptor protein were followed by a significant increase in insulin-stimulated glucose uptake in the isolated mouse heart [24]. The upregulated expression of PDC genes in meldonium-treated animal hearts, together with the observed decrease in lactate concentrations in ischemic hearts, indicate that meldonium treatment may also stimulate aerobic oxidation of glucose [24,41]. Thus, a decrease in L-carnitine content induced by meldonium not only modulates FA metabolism but also stimulates glucose utilization. Altogether, this phenomenon could lead to an optimised balance between glucose and FA oxidation, which is known to be beneficial for the treatment of ischemic heart disease [42]. The meldonium-induced changes in glucose metabolism were attributed to the Randle cycle, as a compensatory mechanism of decreased CPT1-dependent FA oxidation in mitochondria [11,24]. However, an increasing number of observations have noted that the availability of acylcarnitines determines the interplay between FA and glucose metabolism [43] and could thus play a role in the development of insulin resistance [44] and provide novel insight into the mechanisms of the meldonium treatment-induced stimulation of glucose utilization. Diabetic phenotypes have been shown to be induced by increased concentrations of long-chain acylcarnitines in cell culture studies (Fig. 3) [45,46]. Two possible mechanisms are related to an acylcarnitine-induced decrease of pyruvate metabolism in mitochondria [43] and reduced Akt phosphorylation and to insulin signalling-induced glucose uptake via GLUT4 [45]. Therefore, pharmacological interventions that target acylcarnitine accumulation are a novel therapy for the treatment of insulin resistance induced by acylcarnitine accumulation. Overall, a meldonium-induced decrease in acylcarnitine content could be the main mechanism of meldonium's anti-diabetic action (Fig. 5).

3. Activity biomarkers

The long-term administration of meldonium induces distinct changes in biochemical homeostasis, the most important of which
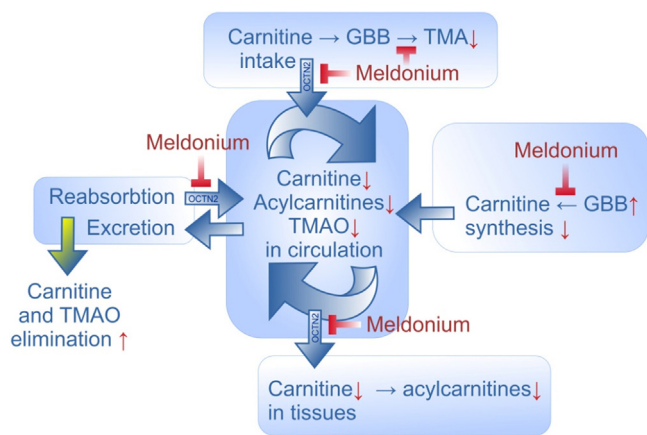
Fig. 4. Effects of meldonium on biomarker concentrations. Meldonium inhibits L-carnitine biosynthesis, and as a result, the concentration of the L-carnitine precursor γ-butyrobetaine (GBB) increases. The inhibition of organic cation/carnitine transporter type 2 (OCTN2) by meldonium (a) reduces the intake of L-carnitine; (b) reduces L-carnitine transport to tissues and the subsequent formation of acylcarnitines; and (c) reduces the renal reabsorption of L-carnitine, thereby stimulating L-carnitine elimination in urine. In addition, meldonium reduces trimethylamine (TMA) formation from GBB by intestinal microbiota and stimulates trimethylamine-N-oxide (TMAO) elimination in urine. Overall, meldonium treatment decreases L-carnitine, acylcarnitine and TMAO concentrations in the circulation.

is the meldonium-induced decrease of L-carnitine [19,38]. Other in vivo metabolic changes can be considered secondary to this effect. The markers of the cardioprotective activity of meldonium include plasma concentrations and tissue contents of L-carnitine, long-chain acylcarnitines, TMAO and γ-butyrobetaine (GBB).

3.1. L-Carnitine

Considering that meldonium acts as an inhibitor of the biosynthesis of L-carnitine (Fig. 4), the decreased tissue content of L-carnitine was mentioned in the first experimental studies as a biochemical marker of cardioprotective activity [11,47]. Adminis-

Fig. 5. Cardioprotective activity of meldonium. The inhibition of organic cation/carnitine transporter type 2 (OCTN2) by meldonium decreases the L-carnitine concentration by reducing L-carnitine transport in tissue and facilitating L-carnitine elimination by urine. The decrease in L-carnitine availability reduces the formation of acylcarnitines by carnitine palmitoyltransferase-1 (CPT1). In addition, meldonium reduces trimethylamine (TMA) formation from L-carnitine by intestinal microbiota and facilitates the elimination of trimethylamine-N-oxide (TMAO), the metabolite of TMA formed by flavin-containing monooxygenases (FMOs). Overall, the decrease in acylcarnitines and TMAO concentrations determines the cardioprotective, anti-atherosclerotic and anti-diabetic effects of meldonium.

tration of a 100 mg/kg dose of meldonium for two weeks induces an approximately 60% reduction in L-carnitine plasma concentration, which is necessary to significantly reduce CPT1-dependent long-chain FA transport and oxidation, as well as to induce subsequent changes in metabolic pathways and a significant reduction in infarct size [19]. L-Carnitine administered with meldonium can diminish both the meldonium-induced decrease in L-carnitine concentration in the tissue and the cardioprotective effect of meldonium [19].

It has been shown that the meldonium-induced (100–400 mg/kg) decrease in L-carnitine is dose-dependent in rats; L-carnitine levels plateau after approximately four weeks of treatment and remain relatively stable thereafter [37,48]. Meldonium treatment decreases the L-carnitine concentration in different organs; this effect has been shown in homogenates of rat heart [47], liver [37], testes [49], brain [50], and aortic tissues [51]. After the administration of an 800-mg/kg dose of meldonium for 10 days, a more than 7-fold decrease in L-carnitine in rat blood plasma and a 20-fold decrease in L-carnitine content in heart tissues can be achieved [23]. Administration of a 30-mg/kg dose of meldonium for two weeks significantly lowers L-carnitine concentrations in rat blood plasma [49], but a 20-mg/kg dose has no effect on these levels [22]. The meldonium treatment-induced reduction of L-carnitine has also been shown in human volunteers; after four weeks of peroral intake of meldonium (at a dose of 500 mg, twice daily), the average L-carnitine concentrations in plasma were significantly decreased by 18% [52]. The consumption of L-carnitine-containing food, particularly meat, partially reduced the meldonium-induced decrease in L-carnitine [52]. Thus far, the reduction of L-carnitine remains the most important and preclinical evidence-based marker of meldonium cardiometabolic activity.

3.2. Long-chain acylcarnitines

L-Carnitine is a substrate in the CPT1-dependent reaction that produces long-chain acylcarnitines and controls the FA flux through the esterification and oxidative pathways [53]. As a result of the meldonium-induced reduction in the L-carnitine concentration, the CPT1-driven reaction produces fewer long-chain acylcarnitines (Fig. 4). The effect of meldonium on long-chain acylcarnitine content was noticed in the first published studies, and the administration of meldonium resulted in decreases in acylcarnitine concentrations of 74% compared to control rat heart tissues [11]. Asaka et al. found that during ischemia, the myocardial long-chain acylcarnitine content increases approximately 7-fold and that meldonium reduced the long-chain acylcarnitine accumulation in hypoxic hearts by 50% [41]. After treatment with a 200-mg/kg dose of meldonium 6 weeks, the plasma concentration of long-chain acylcarnitines was reduced 5-fold [54]. Meldonium was also shown to prevent the accumulation of long-chain acylcarnitines induced by ischemia in an isolated heart model, and it was suggested that preventing the accumulation of long-chain acylcarnitines may be responsible for the cardioprotective effects of meldonium [48]. Additionally, after meldonium treatment, the mitochondrial palmitoylcarnitine content was significantly decreased both in areas at risk and in areas not at risk in the heart [25]. Recently, it has been shown that in long-chain acyl-CoA dehydrogenase (−/−) mice, meldonium treatment eliminates the accumulation of acylcarnitines and improves lung function [55]. Taking into account the regulatory role of long-chain acylcarnitines in energy metabolism pathways and insulin signalling, the effects of meldonium on long-chain acylcarnitine contents might be of interest for future drug discovery projects.
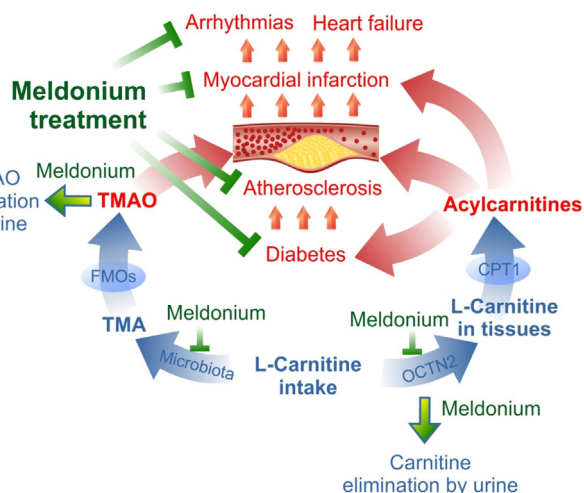
3.3. GBB

Concomitantly with decreased L-carnitine concentration, meldonium treatment increases the concentration of GBB, a substrate of BBOX (Fig. 4). Thus, 100-, 200- or 400-mg/kg doses of meldonium for up to 3 months can induce approximately 10-fold increases in the GBB concentration in the plasma, heart and liver tissues of rats [37]. Administration of 100 mg/kg of meldonium causes a 6-fold increase in GBB concentration in the heart tissues [19]. Although it was first suggested that the GBB concentration that is significantly increased by meldonium treatment is correlated with the cardioprotective effects of meldonium [47], it was later shown that the meldonium-induced reduction of the L-carnitine concentration is the key mechanism of action for the anti-infarction activity of meldonium [19]. The simultaneous administration of meldonium and L-carnitine is needed to achieve a maximal increase in GBB concentrations in vivo. Thus, treatment with L-carnitine alone induced only a 2-fold increase in the GBB concentration, whereas treatment with meldonium or the combination of both substances increased GBB concentrations by up to 10- and 20-fold, respectively [19]. Because the physiological roles of GBB other than its role as a precursor to L-carnitine are not well established, the increased GBB concentration resulting from treatment with meldonium and with a combination of meldonium and L-carnitine remains to be further explored in the future.

3.4. TMAO

TMAO is a metabolite generated from choline and L-carnitine as a result of gut microbiota-dependent metabolism [56,57]. Recently, it has been shown that plasma L-carnitine levels in patients with high TMAO concentrations predict an increased risk of cardiovascular disease [28]. Meldonium is the first low molecular weight, non-antibiotic pharmacological agent shown to decrease the concentration of TMAO in human plasma through increased urinary excretion; the plasma concentration of TMAO in healthy volunteers increased by an average of 16-fold after 7 days of consuming a TMA-rich diet, and meldonium treatment significantly prevented this increase [58]. Later, it was found that meldonium significantly decreases the intestinal microbiota-dependent production of TMA/TMAO from L-carnitine, but the detailed molecular target for this effect is not clear [20]. The TMAO-lowering effect of meldonium is not related to its activity on OCTN2 [58]. Taking into account increasing interest surrounding TMAO as a marker of cardiometabolic health, the effect and involved mechanisms of meldonium might be useful in defining novel drug targets.

4. Pharmacological activity of meldonium

4.1. Cardiovascular diseases

Meldonium is primarily known as a cardioprotective drug whose mechanism of action is based on decreasing the L-carnitine concentration, regulating energy metabolism and enhancing the preconditioning-like adaptive responses [38]. In past years, the cardioprotective effects of meldonium have been extensively studied in different models of cardiovascular diseases [32,39,47,51,69]. The results have demonstrated that meldonium exerted cardioprotective activity against different cardiovascular pathologies through various molecular mechanisms (Fig. 5; Table 2). It was shown that long-term meldonium treatment preserved ATP production by optimizing energy metabolism during hypoxia [11,25,72]. A significant reduction of the infarct size was shown in a rat isolated heart infarction model in vitro [47] after 2 weeks of treatment and in vivo [69] after 10 days of treatment. Moreover, the administration of meldonium exerted cardioprotective effects in diabetic Goto-Kakizaki rats, where, besides the glucose-reducing effect, 100- and 200-mg/kg doses of meldonium decreased the infarct size by 30% [68]. Overall, meldonium treatment effectively reduces myocardial infarction in diabetic Goto-Kakizaki and non-diabetic Wistar rats. Later, it was found that the anti-infarction effect is directly linked to a reduction of L-carnitine pools in cardiac tissues, which further decreases FA transport and protects the outer mitochondrial membrane in heart mitochondria [19].

Meldonium treatment did not demonstrate any significant effect on haemodynamic parameters either before or during ischemia reperfusion, indicating that the observed effects on the infarct size are not related to changes in the cardiac workload [19,47]. This result provides evidence that meldonium primarily acts as a metabolic regulator and provides opportunities to effectively combine meldonium with agents affecting haemodynamic parameters. In animal studies, meldonium was combined with orotic acid, which is a metabolite known to influence energy metabolism [67]. Meldonium possesses additive cardioprotective effects with orotic acid, and meldonium orotate might be considered a powerful therapeutic agent with which to facilitate recovery from ischemia-reperfusion injury. In the same study, we found that the administration of meldonium and its orotate salt decreased the duration and incidence of arrhythmias in experimental arrhythmia models.

The effects of meldonium treatment on haemodynamics, cardiac remodelling and metabolic consequences in rat experimental models of heart failure were also studied [33]. In the experimental model of an aortocaval shunt, it was demonstrated that meldonium treatment attenuated the development of left ventricular (LV) hypertrophy and reduced the increase of LV end diastolic pressure compared to control animals [65]. In a study performed by Hayashi et al., the effects of meldonium were examined in rats with congestive heart failure induced by myocardial infarction [64]. Ventricular remodelling, cardiac function, and myocardial high-energy phosphate levels after 20 days of treatment were measured, and a survival study was conducted for 6 months. Meldonium prolonged survival, prevented the expansion of the left ventricular cavity (ventricular remodelling), attenuated the rise in right atrial pressure and augmented cardiac functional adaptability against an increased load. Additionally, an improved myocardial energy state in meldonium-treated rats was observed. A different study by the same authors demonstrated that the meldonium mechanisms of action in heart failure are based on the amelioration of $[Ca^{2+}]i$ transients through an increase in sarcoplasmic reticulum Ca^{2+} uptake activity [32].

Several clinical studies have evaluated the efficacy of meldonium in the complex therapy of chronic heart failure (CHF). Statsenko et al. evaluated the clinical efficacy of meldonium in addition to basic therapy in patients with CHF and type 2 diabetes mellitus during the postinfarction period [73]. The use of meldonium in addition to basic therapy was associated with a more evident decrease in CHF functional class, an increase in the 6-min walking test distance, a tendency towards the normalization of diastolic heart function and an increase in the left ventricular ejection fraction. Additionally, in a recent study, the advantage of treatment with meldonium (1 g/day) in combination with standard therapy for the exercise tolerance of patients with chronic coronary heart disease over treatment with a placebo in combination with standard therapy was noted [74]. The overall conclusion from clinical studies is that the use of meldonium in basic therapy favours the normalization of vegetative homeostasis and improves quality of life.

Atherosclerosis and the resulting cardiovascular diseases are the main cause of death in the developed world, accounting for one-third of all deaths. Effects of meldonium treatment on the

Table 2
Cardiovascular effects of meldonium in preclinical ex vivo and in vivo models.

Experimental model	Dosing	Effects	References
Isoproterenol- and hydrogen peroxide-induced mechanical and metabolic derangements of rat hearts	100 mg/kg, 7–10 days, i.p./p.o.	Preserved NADH-cytochrome c reductase and cytochrome c oxidase activities; attenuated H_2O_2-induced metabolic derangement; did not affect mechanical heart dysfunction	[59,60]
Myocardial ischemia induced by LAD ligation in rats and dogs	50–200 mg/kg, 10–20 days p.o.	Protected against left ventricular dysfunction; attenuated the derangement of the energy metabolism in the ischemic myocardium; decreased the incidence of ventricular fibrillations	[32,61,62]
Myocardial damage induced by hypoxic perfusion in isolated guinea pig and rat hearts	100 mg/kg, 10 days, p.o.	Improved functional heart parameters, preserved respiratory function of heart mitochondria after hypoxia; prevented the decrease of high-energy phosphates	[41,63]
Cardiac hypertrophy after an aortocaval shunt and heart failure after LAD occlusion	5–100 mg/kg, 3–24 weeks after surgery, p.o.	Attenuated LV hypertrophy development and preserved heart function; prolonged survival; attenuated ventricular remodelling and the rise of right atrial pressure; improved the myocardial energy state	[64,65]
Ischemia/reperfusion-induced arrhythmias in dogs and rats	100 mg/kg, 10–14 days, p.o.	Showed tendency to suppress reperfusion-induced ventricular fibrillation in dogs; decreased the duration and incidence of arrhythmias in rats	[66,67]
LAD occlusion-induced myocardial infarction in ex vivo and in vivo models	100–200 mg/kg, 10–28 days, i.p./p.o./s.c.	Decreased the infarct size after 14 days of treatment; anti-infarction activity was linked to lowered L-carnitine pools; cardioprotective effects were observed in diabetic rats; reduced myocardial infarct size was seen without any effect on haemodynamics in vivo models	[19,47,67–69]
Atherosclerosis development in ApoE/LDLR $^{-/-}$ mice	30 and 100 mg/kg, 4 months p.o.	Attenuated the development of atherosclerosis; decreased L-carnitine content in aortic tissues	[51]
Hypertension and high glucose-induced endothelial dysfunction	Meldonium and its combination with L-carnitine 100 and 100 + 100 mg/kg, respectively, 2–8 weeks, p.o.	Treatment with a combination of L-carnitine and meldonium decreased mortality and attenuated development of hypertension and high glucose-induced endothelial dysfunction	[70,71]

LAD—left anterior descending coronary artery.

development of atherosclerosis were studied in ApoE/LDLr double knockout mice, which received meldonium for four months. The results revealed that meldonium treatment significantly attenuated the development of atherosclerotic lesions in the whole aorta and in the aortic sinus [51]. Later, it was suggested that the anti-atherosclerotic mechanism of meldonium is based on its ability to decrease TMAO levels [20,58]. Vasoprotective effects of meldonium have been observed in several experimental models. It was found that the simultaneous administration of meldonium and L-carnitine, parallel to reduced mortality, attenuated the development of hypertension-induced endothelial dysfunction in salt-sensitive Dahl rats fed a high-salt diet [70]. Moreover, the concomitant administration of meldonium and L-carnitine protected the endothelium of aortic rings against high glucose-induced endothelial dysfunction [71]. It has been hypothesized that one of the possible mechanisms for the vasoprotective effects of meldonium and its combination with L-carnitine could be due to elevated vascular tissue levels of GBB [70,71].

4.2. Metabolic syndrome and diabetes

Administration of meldonium at doses used to induce cardioprotective effects or acute meldonium treatment had no influence on blood glucose levels in non-diabetic rodents [24,37], but it was shown that after 10 days of treatment, an 800-mg/kg dose of meldonium reduced blood glucose by 31% in fasted Wistar rats [75]. Additionally, the long-term administration of a 200-mg/kg dose of meldonium reduced fed state blood glucose levels by increasing glucose uptake and glucose metabolism-related gene expression in the mouse heart [24].

This finding warranted further studies in animals with impaired glucose metabolism. The effects of long-term meldonium treatment on glucose homeostasis, metabolic syndrome development and diabetes are summarized in Table 3. The anti-diabetic effects of meldonium were studied in Goto-Kakizaki rats, an experimental model of type 2 diabetes [68]. The study observed that the decreased availability of L-carnitine by meldonium was accompanied by dose-dependent reductions in blood glucose levels in fed and fasted states without increasing insulin concentrations. Meldonium treatment also prevented diabetes-related endothelial dysfunction and the loss of pain sensitivity [68], thus showing the benefits of meldonium in an experimental model of type 2 diabetes. Similarly, the administration of meldonium for 6 weeks improved glucose tolerance, attenuated the increase of blood glucose and glycated haemoglobin levels in streptozotocin-induced type 1 diabetic rats [76], and prevented the development of streptozotocin-induced diabetic neuropathy [77]. Thus, these studies provide evidence that meldonium treatment reduces hyperglycaemia in experimental models of type 1 and type 2 diabetes mellitus.

Metabolic effects of meldonium treatment have been compared with metformin, a widely used drug for the treatment of type 2 diabetes patients with obesity, in experimental models of obesity and insulin resistance [40]. Meldonium treatment, similarly to metformin treatment, reduced fed and fasted blood glucose levels, and both drugs had a tendency to reduce the elevated insulin concentration in obese Zucker rats. Moreover, the combination of drugs had a synergistic effect on the reduction of insulin concentrations and on

Table 3
Effects of meldonium administration on glucose homeostasis, metabolic syndrome development and diabetes.

Experimental model	Dosing	Effects	References
High-dose test in Wistar rats	800 mg/kg 10 days, p.o.	Decreased blood glucose concentration in the fasted state	[75]
Glucose uptake in isolated mice hearts	200 mg/kg, 20 days, i.p.	Decreased L-carnitine content and subsequently decreased blood glucose levels; increased glucose uptake and glucose metabolism-related gene expression in cardiac tissues	[24]
Development of type 2 diabetes mellitus in Goto-Kakizaki rats	100 and 200 mg/kg, 8 weeks, p.o.	Decreased blood glucose concentration; prevented diabetes-related endothelial dysfunction and the loss of pain sensitivity; decreased L-carnitine content	[68]
Metabolic syndrome model in obese Zucker rats	200 mg/kg, 4 weeks, p.o.	Improved adaptation to hyperglycaemia- and hyperlipidaemia-induced metabolic disturbances; increased PPAR-α activity	[40]
Streptozotocin-induced type 1 diabetes mellitus in rats	100 mg/kg, 6 weeks, p.o.	Normalized blood glucose and glycated haemoglobin concentrations; improved glucose tolerance; prevented the development of diabetic neuropathy	[76,77]

increased liver glycogen, suggesting a significant improvement in liver insulin sensitivity. In contrast to monotherapy, treatment with the combination of drugs significantly prevented weight gain. Thus, the additional increase in body insulin sensitivity and prevention of weight gain are benefits of combination treatment with meldonium and metformin. In addition, a well-known side effect of metformin is increased lactate level. Meldonium treatment alone or in combination with metformin reduced the plasma lactate concentration in obese Zucker rats; therefore, meldonium used in combination could reduce the risk of metformin-induced lactate acidosis. This study concluded that the combination of meldonium and metformin has potential therapeutic value for treating hyperglycaemia- and hyperlipidaemia-induced insulin resistance [40].

The meldonium-induced effects on carbohydrate metabolism is related to a reduction in acylcarnitine availability (Fig. 5), as recent studies suggest that long-chain acylcarnitines are implicated in the development of insulin resistance and diabetes [44]. Thus far, meldonium is the only known clinically used drug that reduces acylcarnitines, and the mechanism of action of meldonium enables it to be combined with other anti-diabetic medications to improve insulin sensitivity.

5. Conclusion

Long-term meldonium treatment lowers L-carnitine contents and induces adaptive effects by causing changes in energy metabolism pathways and in the concentrations of the cardiometabolic risk markers acylcarnitines and TMAO, which are beneficial in the treatment of heart diseases and diabetes. L-carnitine homeostasis is a valuable target for the regulation of energy metabolism pathways and for the treatment of metabolic diseases.

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