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Elevated vascular γ-butyrobetaine levels attenuate the development of high glucose-induced endothelial dysfunction

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SUMMARY

1. The aim of the present study was to investigate the effects of vascular tissue levels of L-carnitine and its precursor, γ-butyrobetaine (GBB), on the development of endothelial dysfunction induced by 5 μmol/L lysophosphatidylcholine (LPC), 10 mmol/L triglycerides (TG) or a high glucose concentration (44 mmol/L).

2. Changes in vascular tissue levels of L-carnitine and GBB were induced by administration of L-carnitine (100 mg/kg), mildronate (100 mg/kg; an inhibitor of L-carnitine synthesis) or their combination to male Wistar rats for 2 weeks.

3. Treatment with L-carnitine elevated vascular tissue levels of L-carnitine, whereas administration of mildronate reduced L-carnitine levels and increased GBB levels. Experimental animals that received the combination of both drugs showed elevated tissue levels of GBB.

4. The results from organ bath experiments demonstrated that increased GBB levels with preserved L-carnitine content in vascular tissues attenuated the development of endothelial dysfunction induced by high glucose. However, changes in vascular tissue L-carnitine and GBB levels had no impact on endothelial dysfunction induced by TG or LPC.

5. The results demonstrate that increased levels of GBB with preserved L-carnitine content in vascular tissue attenuate the development of endothelial dysfunction induced by high glucose concentrations.

Key words: γ-butyrobetaine, L-carnitine, endothelial dysfunction, mildronate, vasoprotection.

INTRODUCTION

L-Carnitine is a molecule that is involved in the energy metabolism of muscle cells and is necessary for long-chain fatty acid influx into the mitochondria, where β-oxidation takes place. In mammals, L-carnitine can be obtained through the diet or synthesized in the liver and kidneys from its precursor γ-butyrobetaine (GBB) by GBB dioxygenase.[1]

Several studies have shown that reduction in the amount of L-carnitine in cardiac tissues is a promising approach to protect the heart against ischaemia–reperfusion injury in healthy, as well as diabetic, rats.[2,3] It has been demonstrated that reductions in tissue levels of L-carnitine attenuate the metabolism of fatty acids[4] and simultaneously facilitate glucose metabolism.[5] It has been proposed that this shift of cellular energy metabolism is responsible for the cardioprotective effects.[2] In these studies, tissue levels of L-carnitine were reduced by the administration of mildronate, which is an inhibitor of both the synthesis and renal reabsorption of L-carnitine.[6] Increased tissue levels of GBB were observed along with the reduction in the amount of L-carnitine.[2,3] γ-Butyrobetaine is a substrate for the last step of the synthesis of L-carnitine.[1] Moreover, some experimental data indicate that GBB may have other functions and properties. For example, it has been shown that GBB itself possesses cardioprotective activity.[7]

In previous studies we showed that decreased L-carnitine and increased GBB levels in vascular tissues induced anti-atherosclerotic effects in an experimental model of atherosclerosis[8] and that marked elevations of GBB in the plasma could attenuate the development of endothelial dysfunction in an experimental model of hypertension.[9] These findings suggest that changes in vascular tissue levels of L-carnitine and GBB could induce vasoprotective effects.

Therefore, the aim of the present study was to investigate the effects of changing vascular tissue levels of L-carnitine and GBB on the development of endothelial dysfunction induced by high concentrations of glucose,[10] triglycerides (TG)[11] or lysophosphatidylcholine (LPC).[12] Each of the substances used was chosen because they represent aetiological factors for the development of endothelial dysfunction in different diseases. Elevated glucose concentrations are linked to the impairment of endothelial function in diabetes;[13] hypertriglyceridaemia is involved in the development of endothelial dysfunction in experimental models of metabolic syndrome;[14] and LPC is a lipid constituent in oxidized low-density lipoproteins, which are involved in the development of endothelial dysfunction in atherosclerosis.[15] Concentrations of substances that induce impairment of function of vascular endothelium were chosen similar or close to those that have been observed in corresponding animal models of the disease.[16,17] Alterations in vascular tissue levels of L-carnitine and GBB were induced by the administration of L-carnitine, mildronate (an inhibitor of L-carnitine biosynthesis) or their combination to male Wistar rats for 2 weeks.

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METHODS

Chemicals

Mildronate dihydrate was obtained from JSC Grindeks (Riga, Latvia). Ketamine hydrochloride solution (Bioketan) was obtained from Vetoquinol Biowet (Gorzów Wielkopolski, Poland) and xylazine hydrochloride solution (Seton) was from Laboratorios Calier (Barcelona, Spain). Acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). L-Carnitine, sodium chloride, potassium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, sodium hydrogencarbonate, potassium dihydrogenphosphate, glucose, EDTA, L-phenylephrine hydrochloride, acetylcholine chloride, ammonium acetate, lysophosphatidylcholine, sodium nitroprusside (SNP), mannitol and collagenase type IV were purchased from Sigma (Schnelldorf, Germany). Intralipid was obtained from Fresenius Kabi (Bad Homburg, Germany).

Experimental animals

Male Wistar rats (7–8 weeks old), weighing 200–240 g, were obtained from the Laboratory of Experimental Animals, Riga Stradins University (Riga, Latvia) and housed under standard conditions (21–23°C, 12 h light–dark cycle, relative humidity 45%–65%) with unlimited access to standard pelletted rat chow (R3 diet; Lactamin, Kimstad, Sweden) and water.

The experimental procedures were performed in accordance with the guidelines of the European Community as well as local laws and policies, and were approved by the Latvian Animal Protection Ethics Committee of the Food and Veterinary Service, Riga, Latvia.

Treatment and experimental protocol

Rats were adapted to the new housing conditions for 1 week prior to treatment. For each experimental protocol, 24 experimental animals were used. Rats were randomly separated into four experimental groups. Experimental animals in the first group received drinking water (control group; $n = 6$), rats in the second group received 100 mg/kg per day mildronate dissolved in the drinking water ($n = 6$), rats in the third group received L-100 mg/kg per day carnitine dissolved in the drinking water ($n = 6$) and rats in the fourth group received a combination of mildronate and L-carnitine (100 mg/kg per day each dissolved in the drinking water; $n = 6$) for 2 weeks. The dosing of mildronate, L-carnitine and their combination was confirmed by measuring the consumption of drinking water every 2 days and adjusting the concentration of supplemented substances.

Until now, administration of a combination of L-carnitine and mildronate is the only known way to elevate GBB levels without decreasing L-carnitine levels.[9] The doses of L-carnitine and mildronate in the combination group were chosen based on the results of a previous study[9] in which administration of the same combination attenuated the development of endothelial dysfunction in an experimental model of hypertension and simultaneously elevated plasma GBB concentrations. The dose of mildronate used herein was chosen on the basis of results from previous studies in which 100 mg/kg mildronate exhibited cardioprotective[3] and vasoprotective[8] effects. In the group receiving L-carnitine, the dose of L-carnitine used was the same as in the combination group to differentiate the effects of L-carnitine from the effects of combination drug treatment.

The experimental animals were anaesthetized with an intraperitoneal injection of a mixture of 100 mg/kg ketamine and 10 mg/kg xylazine 24 h after the last administration of the test compounds. After the onset of anaesthesia, the chest was opened and blood was withdrawn from the right ventricle using a syringe and then transferred into a test tube containing heparin. Plasma samples were prepared by centrifugation at 1000 *g* for 10 min at 4°C. The plasma samples were stored at −80°C until further analysis.

The thoracic aorta was excised and used to measure tissue levels of L-carnitine, GBB and mildronate, or for the assessment of endothelial function in an isolated aortic ring model.

One author (RV) blinded to the identity of the treatment groups performed all the experimental procedures and analyses.

During the study, two experimental animals were excluded from the further experiments due to the development of lung cancer. In addition, results from one experimental animal were excluded from analysis due to unexpected technical problems during organ bath experiments.

Quantification of L-carnitine, GBB and mildronate

The cleaned thoracic aorta was cut into small pieces and incubated in 1 mL Krebs'–Henseleit (K-H) buffer (composition (in mmol/L): NaCl 118; $CaCl_2$ 2.5; $MgCl_2$ 1.64; $NaHCO_3$ 24.88; KH_2PO_4 1.18; glucose 10.0; EDTA 0.05), pH 7.4 at 37°C, containing collagenase type IV (2 mg/mL) until absolute diffluence was achieved. L-Carnitine, GBB and mildronate concentrations in aortic tissue extracts and plasma samples were determined by ultra performance liquid chromatography (UPLC)–tandem mass spectrometry (MS/MS) in positive ion electrospray mode, as described previously.[18] Briefly, the UPLC was performed using an Acquity UPLC system (Waters, Milford, MA, USA) with a Waters Acquity HILIC BEH 1.7 μm 2.1 × 100 mm column; the injection volume was 5 μL. Chromatographic separation was performed in 10 mmol/L ammonium acetate (pH = 4; Solution A) and acetonitrile (Solution B) gradient (0 min 75% Solution B; 2.5 min 55% Solution B; 4 min 50% Solution B) at a flow rate of 0.25 mL/min. The MS/MS analysis was performed on a Micromass Quattro Micro tandem mass spectrometer (Waters). Data were acquired and processed using MassLynx 4.1. software with a QuanLynx 4.1 module (Waters).

Organ chamber experiments

Endothelial function was examined in aortic rings in an organ bath as described previously.[9] The excised thoracic aorta was immersed in ice-cold K-H buffer and cleaned of fatty and connective tissues. The aorta was cut into rings that were 3–4 mm in length. The aortic rings were mounted between two stainless steel hooks in oxygenated K-H buffer solution (pH 7.4, 37°C). The passive tension was fixed at 20 mN. After a period of equilibration (1 h), maximal contractile function was assessed by application of 60 mmol/L KCl. The aortic rings were then precontracted

to 60%–80% of maximal contraction with phenylephrine and a cumulative response curve to acetylcholine (ACh; 10^{-9} to 10^{-5} mol/L) was constructed. After assessment of endothelial function, the aortic rings were incubated in K-H buffer containing agents that impair endothelial function. All the experimental procedures and agents used to induce endothelial dysfunction in the present study have been described previously.[10–12] In the first experimental protocol, endothelial dysfunction was induced by incubation of the aortic rings in a solution containing a high glucose concentration (44 mmol/L) for 3 h. In the second experimental protocol, the function of the vascular endothelium was impaired by incubation of aortic rings in a solution containing a high concentration of TG (10 mmol/L) for 30 min. In the third experimental protocol, endothelial dysfunction was induced by incubation of aortic rings in a buffer containing 5 μmol/L LPC for 30 min After incubation in the presence of the various agents, the aortic rings were precontracted to 60%–80% of maximal contraction with phenylephrine and cumulative response curves to ACh (10^{-9} to 10^{-5} mol/L) were constructed.

To exclude the osmotic effects of glucose on the function of the vascular endothelium, aortic rings from control animals were isolated and endothelial function was assessed before and after incubation in K-H buffer with 33 mmol/L mannitol. In addition, to test the effects of incubation of aortic rings with endothelium dysfunction-inducing agents on vascular smooth muscle function, endothelium-independent relaxation was assessed using SNP.

The relaxation of aortic rings in the presence of acetylcholine or SNP was expressed as the percentage of the phenylephrine-induced constriction and the EC_{50} for each drug was calculated for each aortic ring using Prism 3.0 software (GraphPad, San Diego, CA, USA).

Statistical analysis

Results are expressed as the mean ± SEM. The Kolmogorov–Smirnov test was used to test the distribution of the results obtained. Because the data were normally distributed, the significance of differences between mean values was evaluated using parametric tests. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test as a post hoc test was used to compare the mean amount of L-carnitine, GBB and mildronate in plasma and aortic extract samples. The same test was used to compare EC_{50} values in all four groups before the incubation of aortic rings with endothelial dysfunction-inducing agents. Repeated-measures ANOVA followed by Bonferroni's multiple comparison test as a post hoc test was used to compare responses to ACh before and after incubation of aortic rings with endothelial dysfunction-inducing agents. For the comparison of maximal ACh-induced relaxation (E_{max}) in the LPC-induced endothelial dysfunction model, a paired *t*-test was performed. In addition, paired *t*-tests were used to compare mean EC_{50} values for SNP- and ACh-induced relaxations before and after incubation of aortic rings with mannitol. Two-sided $P < 0.05$ was considered significant.

RESULTS

Analysis of aortic tissue extracts revealed that the administration of mildronate, L-carnitine or their combination induced alterations in vascular tissue levels of GBB and L-carnitine (Fig. 1). In the control group, the average L-carnitine aortic tissue content was 560 ± 40 nmol/g protein. Treatment with mildronate decreased vascular tissue levels of L-carnitine by 52%, but administration of 100 mg/kg L-carnitine for 2 weeks elevated vascular tissue levels of L-carnitine by 36%. Simultaneous administration of mildronate and L-carnitine slightly reduced the L-carnitine content in vascular tissues, but the decrease was not significant.

The average GBB content in vascular tissues of the control group was 40 ± 2 nmol/g protein (Fig. 1b). Treatment with mildronate and the combination of mildronate and L-carnitine resulted in a pronounced increase in GBB levels (3.2- and 3.4-fold increase, respectively). Analysis of the mildronate content revealed that the mean amount of mildronate in vascular tissues in the mildronate-treated group was 410 ± 30 nmol/g protein, whereas in the combination group it was reduced by > 50% (190 ± 10 nmol/g protein).

Treatment of experimental animals with mildronate, L-carnitine or their combination induced changes in plasma GBB and L-carnitine concentrations similar to those seen in aortic tissue (Table 1). The only difference was that the administration of the combination of mildronate and L-carnitine induced a substantially

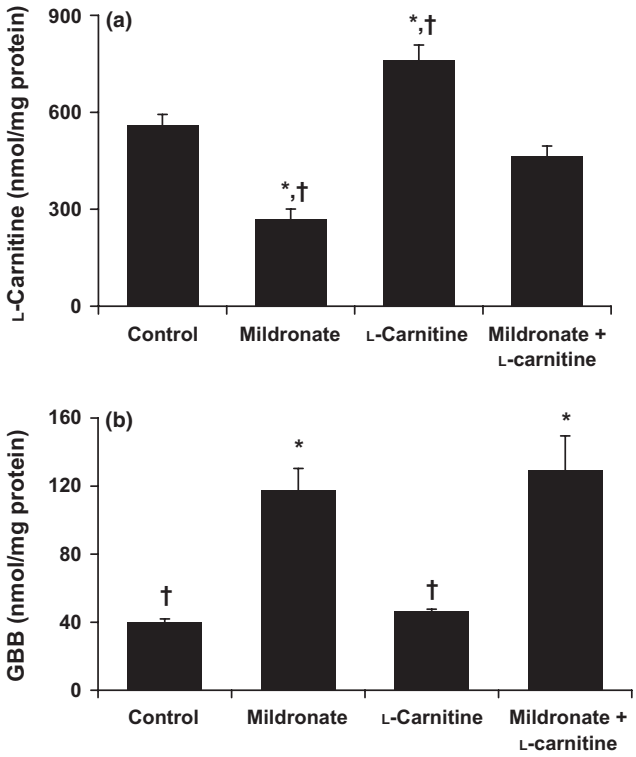

Fig. 1 Vascular tissue levels of (a) L-carnitine and (b) γ-butyrobetaine (GBB) after 2 weeks treatment with mildronate, L-carnitine or their combination. Administration of mildronate decreased L-carnitine levels in vascular tissues, whereas administration of L-carnitine increased L-carnitine content. In addition, treatment of rats with mildronate and the combination of mildronate and L-carnitine increased GBB levels in vascular tissues. Data are the mean ± SEM (n = 5–6 rats). *$P < 0.05$ compared with the control group; †$P < 0.05$ compared with the combination group (one-way ANOVA followed by Tukey's multiple comparison test as a post-test; six multiple comparisons).

Table 1 Plasma concentrations of L-carnitine, γ-butyrobetaine and mildronate after 2 weeks of treatment with mildronate, L-carnitine or their combination

	Carnitine (μmol/L)	GBB (μmol/L)	Mildronate (μmol/L)
Control	48 ± 3	0.6 ± 0.1†	–
Mildronate	22 ± 2*†	6.1 ± 0.9*†	35 ± 9†
L-Carnitine	64 ± 3*†	0.9 ± 0.1*†	–
Mildronate + L-carnitine	50 ± 1	16.0 ± 3.9*	7 ± 1

Data are the mean ± SEM (n = 5–6 rats). *$P < 0.05$ compared with the control group; †$P < 0.05$ compared with the combination group (one-way ANOVA followed by Tukey's multiple comparison test as a post-test; six multiple comparisons).

Administration of mildronate decreased L-carnitine concentration in plasma, whereas administration of L-carnitine increased the amount of L-carnitine in plasma. In addition, treatment of rats with mildronate and the combination of mildronate and L-carnitine increased plasma concentrations of γ-butyrobetaine (GBB).

higher increase in GBB concentrations than the administration of mildronate alone (16.1 ± 4.4 *vs* 6.1 ± 0.9 μmol/L, respectively; $P < 0.05$).

The endothelial effects were evaluated in an isolated thoracic aortic ring model, in which ACh was used to induce endothelium-dependent relaxation. The average EC_{50} values for ACh ranged between 30 and 52 nmol/L (Fig. 2; Table 2) and agreed with those from previous studies.[11,12] Moreover, there were no significant differences in EC_{50} values for ACh among the experimental groups before the incubation of aortic rings with the endothelial dysfunction-inducing agents.

In the control group, incubation of rings in buffer solution containing high concentrations of TG, LPC or glucose resulted in the development of significant impairment of endothelium-dependent relaxation, as evidenced by increased EC_{50} values for ACh (Table 2; Fig. 2) or decreased maximal endothelium-dependent relaxation in response to ACh (Fig. 3).

In the control group, following the incubation of rings in high-glucose buffer solution, EC_{50} values for ACh increased from 39 ± 8 to 81 ± 14 nmol/L ($P < 0.001$). Pretreatment of rats with mildronate or L-carnitine did not result in the development of protection against the development of impaired endothelial function following incubation of rings in high-glucose solution (Fig. 2). The EC_{50} values for ACh before and after incubation of rings in high-glucose solution were 35 ± 4–64 ± 11 nmol/L,

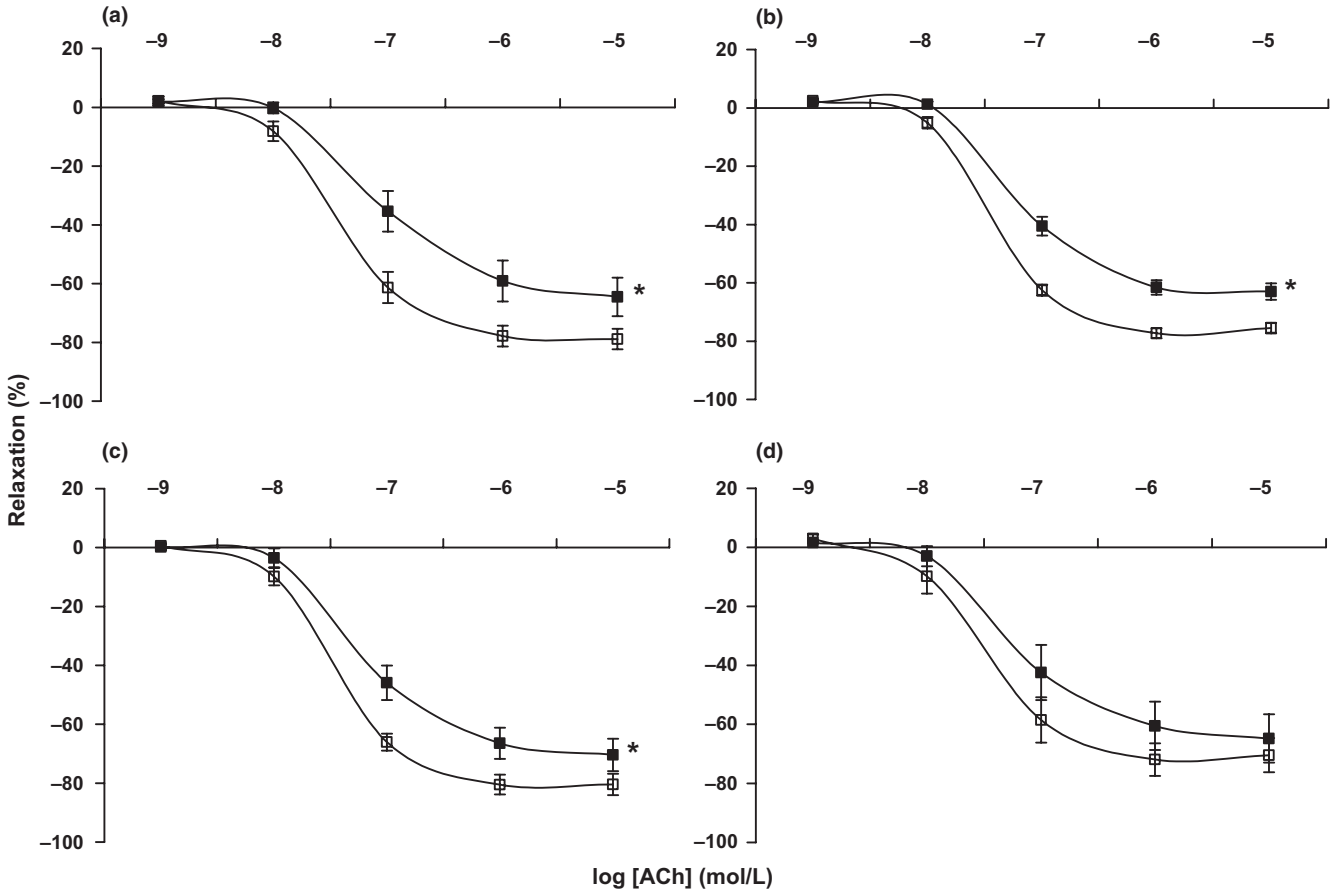

Fig. 2 Effects of the administration of (a) vehicle (water), (b) mildronate, (c) L-carnitine and (d) a combination of mildronate and L-carnitine on the development of endothelial dysfunction induced by a high glucose concentration (44 mmol/L) in buffer solution. (□), responses to acetylcholine (ACh) before incubation with high glucose; (■), responses to ACh after incubation with high glucose. Incubation of aortic rings in buffer solution with high glucose induced impairment of endothelial function. Treatment of rats with mildronate or L-carnitine did not attenuate the development of endothelial dysfunction, whereas administration of the combination did. Data are the mean ± SEM (n = 5–6). *$P < 0.05$ compared with the corresponding response curve before incubation with 44 mmol/L glucose (repeated-measures ANOVA followed by Bonferroni's multiple comparison test as post-test; four multiple comparisons).

Table 2 EC_{50} values for acetylcholine-induced relaxation in aortic rings before and after incubation in buffer solution containing 10 mmol/L triglyceride

	EC_{50} (nmol/L ACh)	
	Before TG	After TG
Control	35 ± 4	294 ± 52*
Mildronate	30 ± 2	370 ± 103*
L-carnitine	35 ± 5	343 ± 61*
Mildronate + L-carnitine	52 ± 10	290 ± 92*

ACh, acetylcholine.

Data are the mean ± SEM (n = 6 rats). *P < 0.05 compared with the corresponding response curve before incubation with 10 mmol/L triglyceride (TG; repeated-measures ANOVA followed by Bonferroni's multiple comparison test as post-test; four multiple comparisons).

Incubation of aortic rings in buffer solution with 10 mmol/L TG induced impairment of endothelial function. None of the pretreatments attenuated the development of endothelial dysfunction.

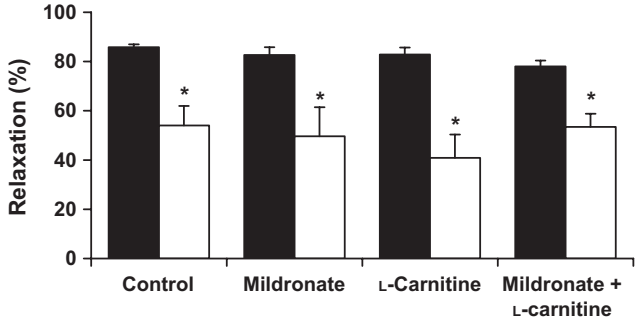

Fig. 3 Effect of administration of vehicle (water), mildronate, L-carnitine or their combination on the maximal relaxation (E_{max}) response induced by acetylcholine before (■) and after (□) incubation of aortic rings with 5 μmol/L lysophosphatidylcholine (LPC). Incubation of aortic rings with 5 μmol/L LPC significantly decreased E_{max} in the control group. None of the treatments used in the study attenuated the impairment of endothelial function. Data are the mean ± SEM (n = 6 rats). *P < 0.05 compared with the corresponding control (paired t-test).

respectively (P < 0.01), in the mildronate group and 32 ± 2–60 ± 8 nmol/L, respectively (P < 0.05), in the L-carnitine group. However, there was no significant impairment of endothelial function in aortic rings from rats treated with the combination of mildronate and L-carnitine, with EC_{50} values for ACh before and after incubation with high-glucose solution being 30 ± 8 and 51 ± 9 nmol/L, respectively (Fig. 2).

Incubation of aortic rings with mannitol for 3 h did not induce any changes in responses to ACh, with EC_{50} values before and after mannitol incubation being 44 ± 5 and 49 ± 5 nmol/L ACh, respectively (n = 6).

The other two endothelial dysfunction-inducing agents impaired endothelial function in aortic rings from rats in the control group. The most prominent impairment of endothelial function was observed after incubation of aortic rings in the presence of 5 μmol/L LPC. In this experiment, endothelial-dependent relaxation did not reach 50% of the phenylephrine-induced submaximal contraction in a subset of aortic rings. Therefore, we did not calculate EC_{50} values, but chose to compare endothelial function by comparing E_{max} values between groups, as reported previously by other researchers using this experimental model.[12] As indicated in Fig. 3 and Table 2, none of the pretreatments attenuated the development of endothelial dysfunction induced by TG or LPC.

Incubation of aortic rings with high glucose concentrations did not alter SNP-induced relaxation, with EC_{50} values before and after incubation being 6.2 ± 1.4 and 4.6 ± 0.8 nmol/L SNP, respectively (n = 6). However, incubation of aortic rings in buffer containing 10 mmol/L TG reduced SNP-induced relaxations, with EC_{50} values before and after incubation with TG being 8.3 ± 0.5 and 43.5 ± 10.9 nmol/L SNP, respectively (n = 7; P = 0.005). In addition, incubation of aortic rings with LPC for 30 min slightly but significantly decreased the E_{max} from 103 ± 1 to 94 ± 2% (n = 6; P = 0.03).

DISCUSSION

In the present study we investigated whether changes in aortic tissue levels of L-carnitine and GBB have vasoprotective effects in experimental models of endothelial dysfunction. In our protocols, endothelial dysfunction was induced by incubation of aortic rings in buffer solutions containing LPC, TG or high glucose. The results showed that administration of the combination of L-carnitine and mildronate, which elevated vascular tissue levels of GBB and had no effect on L-carnitine levels, attenuated the development of endothelial dysfunction induced by high glucose.

Chromatographic analysis of aortic tissue extracts and plasma samples revealed that L-carnitine treatment elevated L-carnitine levels, but that administration of mildronate simultaneously decreased L-carnitine levels and increased GBB levels. Similar changes in vascular tissue and plasma levels of GBB and L-carnitine have been reported previously.[8,9] However, herein we showed, for the first time, that simultaneous administration of L-carnitine and mildronate increases vascular tissue levels of GBB without affecting vascular tissue levels of L-carnitine. This approach allowed us to study the direct effect of elevated GBB levels in vascular tissues on the impairment of endothelial function while excluding the effects induced by changes in L-carnitine levels. In previous studies we found that simultaneous administration of L-carnitine and mildronate caused a more pronounced increase in plasma GBB concentrations than treatment with mildronate alone.[2,9] However, in both the myocardium[2] and vascular tissues (present study), treatment with mildronate or the combination of L-carnitine and mildronate induced similar increased in tissue levels of GBB. Because GBB can be synthesised in various tissues,[19] we propose that the administration of 100 mg/kg mildronate inhibited GBB dioxygenase[4] and increased the tissue content of GBB to the highest pharmacologically attainable level. Moreover, similar conclusions can be drawn from the study in which experimental rats were treated with different doses of mildronate (100, 200 and 400 mg/kg), although the increase in the GBB content in heart tissues was similar across all treatment groups.[20] The differences in GBB plasma levels between groups receiving mildronate and the combination of mildronate plus L-carnitine could be due to the intestinal metabolism of L-carnitine to GBB, which can then be absorbed from the intestinal lumen.[21] Thus, we reason that we have

reached and studied the effects of the highest pharmacologically attainable vascular tissue levels of GBB in our experimental protocol.

Until now not much experimental evidence concerning the vascular effects of GBB has been reported, although elevation of vascular levels of GBB, together with reduced amounts of L-carnitine, has been connected with the anti-atherosclerotic effect of mildronate.[8] However, in several experimental protocols, the methyl ester of GBB has been demonstrated to have ACh-like activity on the vascular endothelium.[22]

Previous studies have shown that the administration of mildronate induced vasoprotective effects in experimental models of atherosclerosis[8] and Type 2 diabetes.[3] Although treatment with mildronate induced expected changes in vascular tissue levels of GBB and L-carnitine in the present study, we did not observe attenuation of the endothelial dysfunction induced by any of the agents that are involved in the development of endothelial dysfunction in atherosclerosis, diabetes and metabolic syndrome. Thus, we think that the vasoprotective effects of mildronate may be the result of a different molecular mechanism or that mildronate treatment in diseased organisms induces different changes than in healthy ones.

Treatment with L-carnitine has been demonstrated to attenuate the development of endothelial dysfunction in different experimental disease models.[23–25] Nevertheless, none of these studies has measured the amount of L-carnitine in vascular tissues or investigated whether the vasoprotective effects of L-carnitine were induced by a direct action of L-carnitine on vascular tissues or whether it is a secondary effect of carnitine supplementation. In our experimental protocol, administration of 100 mg/kg L-carnitine elevated the L-carnitine content in vascular tissue by 36%. However, we did not observe attenuation of the impairment of endothelial function in any of the experimental protocols that we used. This may be explained by the use of a lower dose of L-carnitine compared with that used in other studies that have shown vasoprotective effects of L-carnitine using two- to sixfold higher doses.[23–25]

The administration of a combination of mildronate and L-carnitine attenuated the development of endothelial dysfunction induced by high glucose and, at the same time, elevated the GBB content and had no effect on vascular tissue levels of L-carnitine. We suppose that the vasoprotective effect in this experimental group is due to both elevated GBB levels and unchanged L-carnitine levels. If it were a GBB-dependent effect, we would have also observed vasoprotective effects in the mildronate group. If the effect had been L-carnitine dependent, we would have observed vasoprotective effects in the L-carnitine group. However, we did not observe an attenuation of the development of endothelial dysfunction in either of these two groups.

It has been proposed that the endothelial dysfunction induced by high glucose is mediated through the activation of protein kinase C (PKC).[26] Activation of PKC induces alterations in the prostanoid profile and the generation of reactive oxygen species[27] that could lead to a reduction in nitric oxide (NO) levels and loss of endothelium-dependent vasodilatation. Several studies have demonstrated that NO availability can be increased or that the development of endothelial dysfunction induced by high glucose can be attenuated by inhibition of PKC,[27] increasing heme oxygenase-1 activity[28] or by superoxide anion scavenging molecules.[27] Until now there is no experimental evidence that GBB interacts with any of the previously mentioned proteins. However, it has been shown that direct administration of GBB in rat isolated heart decreases the development of mechanical dysfunction induced by infusion of hydrogen peroxide[7]; thus, the vasoprotective effect of GBB could be due to a reduction in oxidative stress in the vascular wall.

Our results demonstrate that increasing GBB levels while preserving L-carnitine content in vascular tissues attenuates the development of endothelial dysfunction induced by high glucose. This approach could represent a method for the attenuation of the development of endothelial dysfunction in cases of hyperglycaemia, including diabetes.

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DISCLOSURE

The authors declare no conflicts of interest.

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