ELSEVIER

European Journal of Pharmacology 342 (1998) 235–239

ejp

The effect of chronic L-carnitine treatment on blood pressure and plasma lipids in spontaneously hypertensive rats

Hana Rauchová, Zdenka Dobešová, Zdeněk Drahota, Josef Zicha, Jaroslav Kuneš *

Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Prague 4, Czech Republic

Received 5 June 1997; revised 29 August 1997; accepted 4 November 1997

Abstract

The effect of chronic L-carnitine treatment on blood pressure and plasma lipids was studied in spontaneously hypertensive rats (SHR). L-Carnitine treatment for 6 weeks lowered significantly both the systolic and mean arterial pressure of SHR but its influence on diastolic and pulse pressure was only modest. L-Carnitine did not influence the relative heart and kidney weight of SHR. However, L-carnitine completely abolished the age-dependent rise of plasma total cholesterol, triglycerides and uric acid seen in untreated SHR. On the other hand, L-carnitine treatment had no significant effects on blood pressure, relative organ weight and plasma lipids in normotensive Wistar–Kyoto rats. Our results suggest that L-carnitine might prevent some cardiovascular alterations by its influence on lipid metabolism. © 1998 Elsevier Science B.V.

Keywords: Spontaneously hypertensive rat (SHR); Hypertension; L-Carnitine; Cholesterol; Triglyceride; Uric acid

1. Introduction

Carnitine is essential for the transport of long-chain fatty acids from the cytosol to the intramitochondrial space in mammalian cells and thus plays a major role in fatty acid oxidation (Borum, 1983). Some other effects of carnitine and/or its derivatives on cellular metabolism were also demonstrated, e.g. protection against oxygen free radicals (Reznick et al., 1992; Koudelová et al., 1994) or stimulation of mitochondrial biogenesis in aged rats (Gadaleta et al., 1990). Carnitine and its O-acyl-derivatives also influence membrane fluidity, ion channel function, smooth muscle contractility, etc. (Fritz and Arrigoni-Martelli, 1993). It was suggested that membrane effects are implicated in the mechanism by which carnitine derivatives protect the heart from ischaemia or oxidative stress. This might be in concert with findings on the changes of cardiac carnitine metabolism in various hypertensive models.

It was shown that rats subjected to pressure overload (due to aortic constriction) developed cardiac hypertrophy with a reduced myocardial carnitine content but without changes of its serum levels (Reibel et al., 1987). A negative correlation between myocardial carnitine content and the degree of cardiac hypertrophy was demonstrated in this experimental model (Yang et al., 1992). In contrast, carnitine was elevated in hypertrophied hearts of spontaneously hypertensive rats (SHR) aged 10 or 15 weeks and this was associated with elevated serum carnitine levels (Foster et al., 1985). In young animals, in which cardiac hypertrophy and blood pressure were not so high, serum carnitine level did not differ between SHR and normotensive Wistar–Kyoto (WKY) rats (Foster et al., 1985).

In WKY rats with the constriction of abdominal aorta, the treatment with propionyl-L-carnitine, naturally occurring carnitine derivative, restored their cardiac function and carnitine myocardial content to normal values (Yang et al., 1992). Recently, Mauriello et al. (1996) studied the influence of long-term propionyl-L-carnitine treatment in adult SHR. Although this treatment did not lower blood pressure, vascular smooth muscle polyploidy was diminished in the aorta of treated SHR. Chronic propionyl-L-carnitine treatment was also reported to lower plasma triglycerides and cholesterol in aged hyperlipemic rabbits (Spagnoli et al., 1995). Similarly, oral L-carnitine administration decreased plasma triglycerides, cholesterol and phospholipids in rats fed olive oil (Maccari et al., 1987).

* Corresponding author. Tel.: +420-2-4752420; fax: +420-2-4752488.

We studied whether the treatment of SHR with L-carnitine for 6 weeks could influence their blood pressure or cardiac hypertrophy as well as their plasma cholesterol, triglycerides and uric acid levels.

2. Materials and methods

Male spontaneously hypertensive (SHR) and normotensive Wistar–Kyoto (WKY) rats aged 2 months were obtained from the colony of the Institute of Physiology, AS CR (Prague). The rats were kept under standard conditions (23 ± 1°C, 12:12 h light–dark cycle) and were fed a standard pellet diet with free access to tap water. One half of the animals was offered 0.1% L-carnitine solution to drink instead of water. The amount of consumed carnitine was about 0.2 g/kg per day calculated from their daily water consumption. We used the same dose as in our previous experiments (Koudelová et al., 1994). It represents approximately the doubling of daily dietary L-carnitine intake (Borum, 1983) and maintains a plasma level of carnitine approximately three times as high as in control animals.

Every two weeks blood from the tail arteries was taken for the measurement of plasma total cholesterol, triglycerides and uric acid. At the end of the experiment systolic, mean arterial and diastolic blood pressures were determined directly in the carotid artery under light ether anaesthesia. Blood from the aorta was taken for the determination of plasma total cholesterol, triglycerides and uric acid as well as for estimation of serum levels of total, free and esterified carnitine. The relative heart and kidney weights were determined after killing of the animals.

Plasma total cholesterol, triglycerides and uric acid concentrations were determined by commercial kits (Lachema, Brno). Serum levels of total and free carnitine were measured by the method of Wieland et al., 1985.

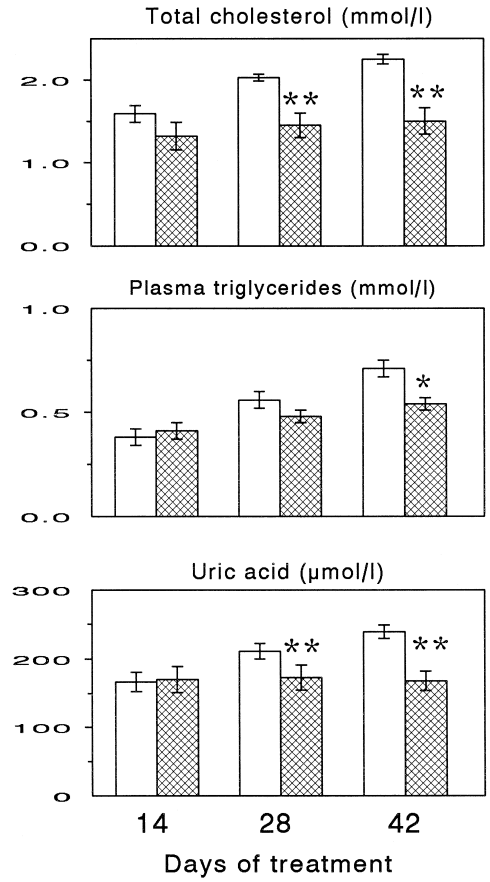

Fig. 1. Age-dependent changes of plasma total cholesterol, triglycerides and uric acid in SHR without (open columns) or with (cross-hatched columns) L-carnitine treatment for 6 weeks. $^{*}P < 0.01$, $^{**}P < 0.001$ different from untreated animals.

The results are expressed as means ± S.E.M. The data were evaluated with one-way ANOVA with a least signifi-

Table 1
Body weight, relative heart (HW/BW) and kidney (KW/BW) weights as well as blood pressure in spontaneously hypertensive (SHR) and normotensive Wistar–Kyoto (WKY) rats. The animals were without (−CARN) or with (+CARN) L-carnitine treatment for 6 weeks

	WKY		SHR		F/LSD
	−CARN	+CARN	−CARN	+CARN	
Number of animals	8	8	9	9	
Body weight (g)	275 ± 14	277 ± 10	225 ± 6[a]	230 ± 2[a]	10.3/25.8
HW/BW (mg/100 g)	271 ± 5	266 ± 2	347 ± 6[a]	342 ± 4[a]	91.5/13.5
KW/BW (mg/100 g)	547 ± 6	535 ± 9	660 ± 12[a]	657 ± 5[a]	62.8/25.3
SBP (mmHg)	144 ± 1	139 ± 2	188 ± 2[a]	177 ± 4[a,b]	86.5/7.7
MAP (mmHg)	120 ± 2	114 ± 4	153 ± 2[a]	145 ± 3[a,b]	44.3/8.4
DBP (mmHg)	100 ± 2	95 ± 4	123 ± 2[a]	118 ± 4[a]	18.3/9.3
PP (mmHg)	44 ± 3	43 ± 3	65 ± 2[a]	59 ± 3[a]	15.8/8.1

Data are means ± S.E.M.
SBP, systolic pressure; MAP, mean arterial pressure; DBP, diastolic pressure; PP, pulse pressure; F, F ratio ($df_1 = 3$, $df_2 = 30$); LSD, least significant difference (at $P < 0.05$ level).
[a] $P < 0.001$ different from WKY.
[b] $P < 0.05$ different from untreated animals.

Table 2
Serum content of total, free and esterified carnitine in spontaneously hypertensive (SHR) and normotensive Wistar–Kyoto (WKY) rats. The animals were without (−CARN) or with (+CARN) L-carnitine treatment for 6 weeks

	WKY		SHR		F/LSD
	−CARN	+CARN	−CARN	+CARN	
Number of rats	8	8	9	9	
Total carnitine	43.4 ± 2.2	150.5 ± 6.6[b]	35.7 ± 1.8	96.9 ± 9.6[d,b]	73.8/18.1
Free carnitine	22.0 ± 2.1	99.5 ± 5.7[b]	21.8 ± 1.9	55.2 ± 4.8[d,b]	83.4/11.6
Esterified carnitine	22.1 ± 0.7	57.5 ± 1.9[b]	16.0 ± 0.9	32.3 ± 7.7[a,c]	18.2/12.4

Data are means ± S.E.M.
F, F ratio ($df_1 = 3$, $df_2 = 30$); LSD, least significant difference (at $P < 0.05$ level).
[a] $P < 0.05$.
[b] $P < 0.001$ different from untreated animals.
[c] $P < 0.01$.
[d] $P < 0.001$ different from WKY.

cant difference test (Snedecor and Cochran, 1968). $P < 0.05$ was considered as significant.

3. Results

L-Carnitine treatment did not influence the body weight of the animals of the two strains (Table 1). In control WKY rats, L-carnitine had no significant effect on blood pressure or relative organ weight. In SHR, on the other hand, L-carnitine treatment decreased significantly both the systolic and mean arterial blood pressure but the influence on diastolic and pulse pressure was only modest. There was also no effect of L-carnitine on relative heart and kidney weight in SHR. L-Carnitine completely abolished the age-dependent rise of plasma total cholesterol, triglycerides and uric acid levels seen in untreated SHR (Fig. 1). Thus, at the end of the experiment there were highly significant differences of all three variables between untreated and carnitine-treated SHR. In contrast, no L-carnitine effect on these metabolic parameters was seen in WKY (data not shown).

The concentration of total and esterified carnitine tended to be lower in untreated SHR than in untreated WKY rats (Table 2). In both strains, the values of total, free and esterified carnitine were always elevated in L-carnitine-treated animals compared to untreated ones, the increase being greater in WKY rats. Consequently, lower concentrations of total, free and esterified carnitine were found in carnitine-treated SHR in comparison with treated WKY rats (Table 2).

4. Discussion

The present study demonstrated that the treatment of SHR with L-carnitine for 6 weeks lowered their blood pressure but it had no effects in normotensive WKY rats. To our knowledge, this is the first report that L-carnitine treatment reduced blood pressure in genetically hypertensive animals. The absence of blood pressure reduction in SHR treated with propionyl-L-carnitine (Mauriello et al., 1996) might be ascribed either to the different metabolism of L-carnitine and propionyl-L-carnitine or to the use of 4-month-old rats with fully established hypertension. However, propionyl-L-carnitine treatment was reported to lower systemic resistance in hypertensive rats with aortic constriction (Yang et al., 1992). A slight decrease of both systolic and diastolic blood pressure was also found in hypertensive humans subjected to long-term L-carnitine treatment in which improved left ventricular function and decreased plasma cholesterol were observed (Digiesi et al., 1994). In our study on SHR, chronic L-carnitine treatment substantially lowered plasma total cholesterol and moderately decreased plasma triglycerides. This is in good agreement with the lipid lowering effect of carnitine in rats fed diets rich in fat or cholesterol (Shimura and Hasegawa, 1993). Chronic L-carnitine treatment of hyperlipidemic fat-loaded rats (Maccari et al., 1987) lowered not only plasma triglycerides and cholesterol but also plasma free fatty acids. This effect of L-carnitine seems to be related to the changes in hepatic fatty acid handling. On the basis of chronic in vivo and acute in vitro experiments it was suggested that L-carnitine administration promotes β-oxidation of fatty acids and thus lowers the hepatic production of very low density lipoproteins (Maccari et al., 1987).

At present we can only speculate about the mechanisms by which L-carnitine can reduce blood pressure in SHR. Membrane effects of carnitine seem to be one of the most probable possibilities of its antihypertensive action.

L-Carnitine and its short-chain acyl derivatives are known to stabilise biomembranes through the interaction with membrane lipid bilayer (Fritz and Arrigoni-Martelli, 1993). Stabilising effects of L-carnitine on membrane fluidity are based upon the prevention of detergent effects of various amphiphilic compounds formed during ischaemia or oxidative stress (Kobayashi et al., 1989). These L-carnitine derivatives affect molecular dynamics of a membrane bilayer close to glycerol backbone of phospholipids, i.e. in the region which is relevant for the expression of various

membrane functions (Arduini et al., 1993). Moreover, carnitine increases membrane stability, most likely via the interaction with cytoskeleton proteins (Arduini et al., 1990). Thus membrane stabilising effects of L-carnitine might diminish membrane disturbances characteristic for genetic hypertension (Dominiczak and Bohr, 1991; Resnick, 1993; Aviv, 1996).

Short-chain acyl derivatives of L-carnitine also prevent lipid peroxidation induced in various cardiovascular tissues by excess of oxygen free radicals (Schinetti et al., 1987; Ferrari et al., 1988; Bertelli et al., 1991). The mechanism of antiradical effect of short-chain acyl derivatives of L-carnitine is non-specific, probably by chelating the iron required for the generation of hydroxyl radicals (Reznick et al., 1992).

The decrease of plasma uric acid level observed in SHR treated with L-carnitine might reflect not only diminished purine degradation but also reduced activity of xanthine oxidase which results in lower formation of oxygen free radicals (Nakazono et al., 1991). The in vivo administration of L-carnitine indeed attenuated lipid peroxidation (Koudelová et al., 1994). The excess of oxygen free radicals seems to be involved in the pathogenesis of genetic hypertension either through its interference with NO-mediated vasodilatation (Grunfeld et al., 1995; Tschudi et al., 1996) or through ion transport alterations resulting from the membrane injury associated with lipid peroxidation (Ito et al., 1993; Koudelová et al., 1994). Short-chain acyl derivatives of L-carnitine are also effective in the protection of vascular endothelium, e.g. against oxidative damage (Bertelli et al., 1991; Hulsmann and Dubelaar, 1992).

It should, however, be mentioned that cardiovascular effects of chronic carnitine administration might substantially differ from its acute in vitro effects because L-carnitine is rapidly esterified in vivo. At least a part of L-carnitine is metabolised to long-chain acyl derivatives because oral treatment of rats with propionyl-L-carnitine substantially improved the incorporation of long-chain fatty acids into membrane phospholipids (Arduini et al., 1995). Long-chain acyl derivatives of L-carnitine often have quite opposite effects than L-carnitine itself. The effects of long-chain derivatives include, for example, the rise in membrane fluidity (Watanabe et al., 1989) or the attenuation of endothelium-dependent vasorelaxation (Dainty et al., 1990; Inoue et al., 1994). A smaller rise in plasma levels of free and esterified carnitine in SHR than in WKY rats suggests that possible differences of carnitine metabolism in both strains would be worth of future investigation.

Further experiments should also be focused on the determination of possible alterations of membrane properties (including membrane microviscosity and cell Ca^{2+} handling) as well as on changes of lipid peroxidation in cardiovascular tissues of normotensive and hypertensive animals subjected to chronic L-carnitine treatment. The role of lipoproteins should also be studied in more details because Maccari et al. (1987) found a decrease of very low density lipoprotein fraction in hyperlipidemic fat-loaded rats treated with L-carnitine.

Acknowledgements

This study was partially supported by research grants 303/95/0615 (Grant Agency of the Czech Republic) and A7011711 (Grant Agency of Academy of Sciences of the Czech Republic). L-Carnitine was a kind gift of Sigma Tau (Milan, Italy).

References

Arduini, A., Rossi, M., Mancinelli, G., Belfiglio, M., Scurti, R., Radatti, G., Shohet, S.B., 1990. Effect of L-carnitine and acetyl-L-carnitine on the human erythrocyte membrane stability and deformability. Life Sci. 47, 2395–2400.

Arduini, A., Gorbunov, N., Arrigoni-Martelli, E., Dottori, S., Molajoni, F., Russo, F., Federici, G., 1993. Effects of L-carnitine and its acetate and propionate esters on the molecular dynamics of human erythrocyte membrane. Biochim. Biophys. Acta 1146, 229–235.

Arduini, A., Dottori, S., Sciarroni, A.F., Corsico, N., Morabito, E., Arrigoni-Martelli, E., Calvani, M., 1995. Effect of propionyl-L-carnitine treatment on membrane phospholipid fatty acid turnover in diabetic rat erythrocytes. Mol. Cell Biochem. 152, 31–37.

Aviv, A., 1996. The links between cellular Ca^{2+} and Na^{+}/H^{+} exchange in the pathophysiology of essential hypertension. Am. J. Hypertens. 9, 703–707.

Bertelli, A., Conte, A., Palmieri, L., Ronca, G., Segnini, D., Yu, G., 1991. Effect of propionyl carnitine on energy charge and adenine nucleotide content of cardiac endothelial cells during hypoxia. Int. J. Tissue React. 13, 37–40.

Borum, R.P., 1983. Carnitine. Annu. Rev. Nutr. 3, 233–259.

Dainty, I.A., Bigaud, M., McGrath, J.C., Spedding, M., 1990. Interactions of palmitoyl carnitine with the endothelium in rat aorta. Br. J. Pharmacol. 100, 241–246.

Digiesi, V., Cantini, F., Bisi, G., Guarino, G., Brodbeck, B., 1994. L-carnitine adjuvant therapy in essential hypertension. Clin. Ter. 144, 391–395.

Dominiczak, A.F., Bohr, D.F., 1991. The primacy of membrane microviscosity in genetic hypertension. Am. J. Hypertens. 4, 963–969.

Ferrari, R., Ciampalini, G., Agnoletti, G., Cargnoni, A., Ceconi, C., Visioli, O., 1988. Effect of L-carnitine derivatives on heart mitochondrial damage induced by lipid peroxidation. Pharmacol. Res. Commun. 20, 125–132.

Foster, K.A., O'Rourke, B., Reibel, D.K., 1985. Altered carnitine metabolism in spontaneously hypertensive rats. Am. J. Physiol. 249, E183–E186.

Fritz, I.B., Arrigoni-Martelli, E., 1993. Sites of action of carnitine and its derivatives on the cardiovascular system: Interactions with membranes. Trends Pharmacol. Sci. 14, 355–360.

Gadaleta, M.N., Petruzzella, V., Renis, M., Fracasso, F., Cantatore, P., 1990. Reduced transcription of mitochondrial DNA in the senescent rat. Tissue dependence and effect of L-carnitine. Eur. J. Biochem. 187, 501–506.

Grunfeld, S., Hamilton, C.A., Mesaros, S., McClain, S.W., Dominiczak, A.F., Bohr, D.F., Malinski, T., 1995. Role of superoxide in the depressed nitric oxide production by the endothelium of genetically hypertensive rats. Hypertension 26, 854–857.

Hulsmann, W.C., Dubelaar, M.L., 1992. Carnitine requirement of vascu-

lar endothelial and smooth muscle cells in imminent ischemia. Mol. Cell Biochem. 116, 125–129.

Inoue, N., Hirata, K., Akita, H., Yokoyama, M., 1994. Palmitoyl-L-carnitine modifies the function of vascular endothelium. Cardiovasc. Res. 28, 129–134.

Ito, H., Torii, M., Suzuki, T., 1993. A comparative study on lipid peroxidation in cerebral cortex of stroke-prone spontaneously hypertensive and normotensive rats. Int. J. Biochem. 25, 1801–1805.

Kobayashi, A., Watanabe, H., Fujisawa, S., Yamamoto, T., Yamazaki, N., 1989. Effects of L-carnitine and palmitoylcarnitine on membrane fluidity of human erythrocytes. Biochim. Biophys. Acta 986, 83–88.

Koudelová, J., Mourek, J., Drahota, Z., Rauchová, H., 1994. Protective effect of carnitine on lipoperoxide formation in rat brain. Physiol. Res. 43, 387–389.

Maccari, F., Arseni, A., Chiodi, P., Ramacci, M.T., Angelucci, L., Hulsmann, W.C., 1987. L-Carnitine effect on plasma lipoproteins of hyperlipidemic fat-loaded rats. Lipids 22, 1005–1008.

Mauriello, A., Sangiorgi, G., Orlandi, A., Schiaroli, S., Perfumo, S., Spagnoli, L.G., 1996. Effect of long-term treatment with propionyl-L-carnitine on smooth muscle cell polyploidy in spontaneously hypertensive rats. Hypertension 28, 177–182.

Nakazono, K., Watanabe, N., Matsuno, K., Sasaki, J., Sato, T., Inoue, M., 1991. Does superoxide underlie the pathogenesis of hypertension?. Proc. Natl. Acad. Sci. USA 88, 10045–10048.

Reibel, D.K., O'Rourke, B., Foster, K.A., 1987. Mechanisms for altered carnitine content in hypertrophied rat hearts. Am. J. Physiol. 252, H561–H565.

Resnick, L.M., 1993. Ionic basis of hypertension, insulin resistance, vascular disease, and related disorders. The mechanism of 'syndrome X'. Am. J. Hypertens. 6, 123S–134S.

Reznick, A.Z., Kagan, V.E., Ramsey, R., Tsuchiya, M., Khwaja, S., Serbinova, E.A., Packer, L., 1992. Antiradical effects in L-propionyl carnitine protection of the heart against ischemia-reperfusion injury: The possible role of iron chelation. Arch. Biochem. Biophys. 296, 394–401.

Schinetti, M.L., Rossini, D., Greco, R., Bertelli, A., 1987. Protective action of acetylcarnitine on NADPH-induced lipid peroxidation of cardiac microsomes. Drugs Exp. Clin. Res. 13, 509–515.

Shimura, S., Hasegawa, T., 1993. Changes of lipid concentrations in liver and serum by administration of carnitine added diets in rats. J. Vet. Med. Sci. 55, 845–847.

Snedecor, G.W., Cochran, W.G., 1968. Statistical Methods, 6th ed. Iowa State University Press, Ames, IA, pp. 258–298.

Spagnoli, L.G., Orlandi, A., Marino, B., Mauriello, A., De Angelis, C., Ramacci, M.T., 1995. Propionyl-L-carnitine prevents the progression of atherosclerotic lesions in aged hyperlipemic rabbits. Atherosclerosis 114, 29–44.

Tschudi, M.R., Mesaros, S., Lüscher, T.F., Malinski, T., 1996. Direct in situ measurement of nitric oxide in mesenteric resistance arteries. Increased decomposition by superoxide in hypertension. Hypertension 27, 32–35.

Watanabe, H., Kobayashi, A., Hayashi, H., Yamazaki, N., 1989. Effects of long-chain acyl carnitine on membrane fluidity of human erythrocytes. Biochim. Biophys. Acta 980, 315–318.

Wieland, O.H., Deufel, T., Paetzke-Brunner, I., 1985. Free and esterified carnitine: Colorimetric method. In: Bergmeyer, H.U. (Ed.), Methods in Enzymatic Analysis. Academic Press, New York, pp. 481–488.

Yang, X.P., Samaja, M., English, E., Benatti, P., Tarantola, M., Cardace, G., Motterlini, R., Micheletti, R., Bianchi, G., 1992. Hemodynamic and metabolic activities of propionyl-L-carnitine in rats with pressure-overload cardiac hypertrophy. J. Cardiovasc. Pharmacol. 20, 88–98.