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Effects of L-Carnitine on the Hypoxic Atria from Fed and Fasted Rats

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The aim of the investigation was to assess whether L-carnitine, an essential cofactor in the mitochondrial transfer of fatty acids, would ameliorate the hypoxic-induced disturbances in the isolated rat atria. During hypoxia, the atria released lactate into the bathing medium and underwent a rise in resting tension and a decline of the peak developed tension and pacemaker frequency. The atria from 24-h fasted rats, which oxidize faster their endogenous triacylglycerol stores» exhibited greater functional disturbances during hypoxia and a smaller recovery after reoxygenation, with respect to the fed rats' atria. Furthermore, at the end of the hypoxic incubation the fasted rats atria displayed a reduction of the free CoA content together with a 3-fold increase in the content of long-chain fatty-acyl CoA, in comparison with those of fed rats. The addition of 5 mM L-carnitine 60 min before the onset of hypoxia did not exert any effect on the hypoxic atria. In contrast, 20 mM L-carnitine accelerated the decline of the pacemaker activity in the fasted rat atria and worsened the contracture development in both nutritional states. The fall of the peak tension and the posthypoxic recovery as well as the levels of free CoA and long-chain fatty-acyl CoA, and lactate output, were not affected by 20 mM L-carnitine treatment. These data suggest that L-carnitine is not beneficial for the hypoxic rat atria, even in the fasted state, wherein the atrial fatty acid catabolism is increased.

Key words: Carnitine, Hypoxia, Fasting, Rat atria, Acyl CoA.

The atria from fasted rats, when compared to those from fed rats, exhibit a faster impairment of their functional properties during hypoxia as well as a smaller recovery after reoxygenation (3, 18, 33). Since the atria from fasted rats have greater triacylglycerol stores and a faster *in vitro* lipolysis (32, 34), the enhanced lability of these atria under hypoxic conditions might be ascribed to

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noxious effects of fatty acids. Hypoxia restrains the fatty acid β-oxidation leading to a cytotoxic accumulation of fatty acids and long-chain fatty-acyl CoA esters (15, 28, 38) which exacerbates the hypoxic-induced disturbances through the inhibition of several critical enzyme systems (9, 11, 16, 26, 27, 41). On the other hand, carnitine, an essential cofactor in the mitochondrial transfer of activated long-chain fatty acids, may be able to reverse these inhibitory effects through attenuating the harmful accumulation of fatty acids and their derivatives (4, 13, 19, 20, 26). In addition, carnitine activates the pyruvate dehydrogenase and improves the flux of pyruvate into the citric acid cycle (1, 19). On these bases, carnitine has been already used to ameliorate the hypoxic and ischemic disturbances. In the dog heart carnitine was reported to decrease the occurrence of electrocardiographic changes and ventricular fibrillation (6, 29,37). Furthermore, in the rat (10, 22, 23) and swine (12) hearts, carnitine improved the mechanical activity during ischemia and reperfusion, although in the swine its effects were manifested only in the presence of excess fatty acids. On the contrary, in other investigations carnitine showed to be ineffective on the hypoxic or ischemic hearts of rat and swine (17, 21, 24) as well as detrimental to the ischemic rat heart (7). Therefore, the effects of carnitine during oxygen-limited conditions remains a controversial issue and deserves further examination. On these bases, we were prompted to carry out an investigation using the isolated rat atria, whose metabolic needs do not rely on coronary circulation. Thus, any action of carnitine effected through changes of the coronary flow, would be avoided. Furthermore, in order to assess the influence of fatty acid catabolism, responses to atria from fed and fasted rats were compared.

Materials and Methods

Female Wistar rats weighing 200-250 g, maintained on a 12-h dark/light cycle, either fed *ad libitum* or fasted 24-h, were decapitated. The atria were quickly excised and mounted isometrically at 750 mg of resting tension. The bathing medium was a Krebs Ringer bicarbonate solution containing 1.6 mM Ca and 11 mM glucose, kept at 31 °C and continuously bubbled with 95 % O_2 - 5 % CO_2. The contraction frequency of spontaneously beating atria were measured from 30 s samples of the recorded contractions. Peak developed tension and changes of resting tension were measured in left atria paced at 1 Hz with 5-10 V, 0.6 ms square pulses. Carnitine (L-carnitine HCl, Sigma) was dissolved in water and added 15 min after mounting the atria. The pH of the solution was adjusted to 7.4 with $NaCO_3H$ and the NaCl of the bathing medium was equimolarly reduced in order to maintain unchanged the Na concentration. Hypoxia started 1-h after adding carnitine (5 or 20 mM) by bubbling the organ-bath with N_2 instead of O_2.

The lactate released into the medium was assayed using the HOHORST's (8) enzymatic procedure. Long-chain acyl CoA and free CoA were extracted according to the method described by WILLIAMSON and CORKEY (39) and acyl CoA was hydrolyzed at pH 11.5 - 12.0, at 55 °C during 15 min. CoA was measured by means of the VELOSO and VEECH (36) enzymatic cycling method. The release of lactate and the atrial content of free CoA and acyl CoA were statistically compared by a two factors ANOVA followed by the Tukey's test; changes of the contractions frequency, peak developed tension and resting tension using a three factors ANOVA for repeated measures in one factor followed by the Tukey's test (40).

Results

Over the 60 min hypoxic incubation, paced left atria displayed a pronounced depression of their peak developed tension. The extent of the decline was similar in the presence of 5 or 20 mM carnitine (data not shown). Simultaneously, the left atria developed a contracture, as indicated by a rise in resting tension at constant muscle length. Agreeing with previous data (33), this rise was greater in the atria from fasted rats. Five mM carnitine did not alter the contracture strength (data not shown) whereas 20 mM carnitine increased it in the atria of both nutritional states (fig. 1). In another experimental design, 30 min after the onset of hypoxia a 30 min recovery period was allowed by reoxygenating the organ-bath. As illustrated in fig. 2, the fed rats atria attained a higher recovery of the peak tension than those from fasted rats and 20 mM carnitine did not exert any effect in both of the experimental groups.

The contraction frequency declined progressively throughout the hypoxic incubation, the decline being greater in the atria from fasted rats. Five mM carnitine had no effect (data not shown) whereas in the group of fasted rats 20 mM carnitine accelerated the decline during the early 20 min of hypoxia (fig. 3).

Data recorded in table I indicate that, as compared with the fed rats, the atria from fasted rats display a 3-fold increase in the content of long-chain fatty-acyl CoA at the end of the hypoxic incubation, where-

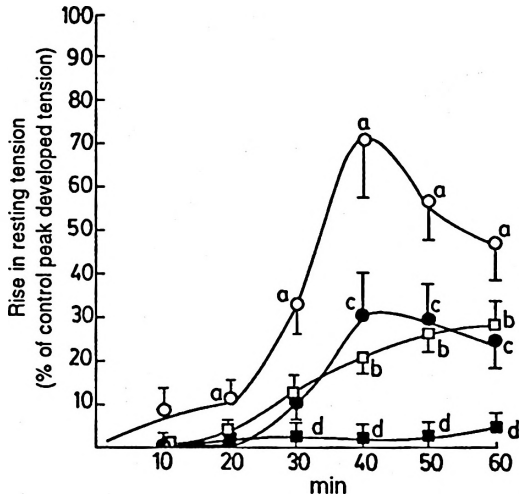

Fig. 1. *Rise in resting tension of left atria paced at 1 Hz, during hypoxic incubation.*
Squares: fed rats. Circles: 24-h fasted rats. Closed symbols: normal medium. Open symbols: medium containing 20 mM L-carnitine, added 60 min before the onset of hypoxia. Zero time refers to the end of the prehypoxic incubation. Values are the average of 7 atria ± S.E.M. a: $p < 0.01$ vs the fed rat atria in the carnitine containing medium. b: $p < 0.01$ vs the fed rat atria in the normal medium. c: $p < 0.01$ vs the fasted rat atria in the carnitine containing medium. d: $p < 0.01$ vs the fasted rat atria in the normal medium.

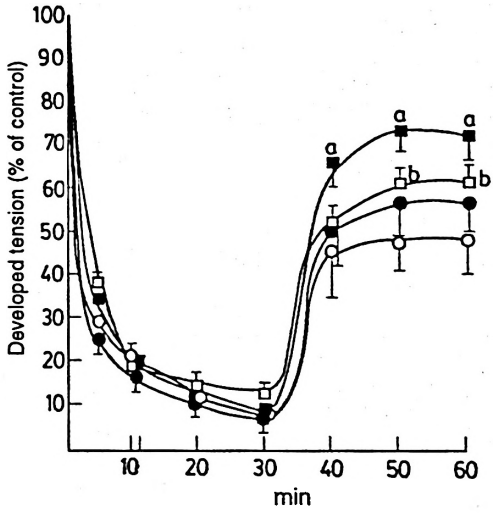

Fig. 2. *Effects of hypoxia and reoxygenation on the peak developed tension of left atria paced at 1 Hz.*
Symbols as in fig. 1. Peak developed tension at the end of the prehypoxic incubation (mg): fed rats in control medium, 390 ± 61; fed rats in the carnitine containing medium, 343 ± 48; fasted rats in the normal medium, 450 ± 105; fasted rats in the carnitine containing medium, 413 ± 46. Values are the average of 7 atria ± S.E.M. a: $p < 0.01$ vs the fasted rat atria in the normal medium. b: $p < 0.01$ vs the fasted rat atria in the carnitine containing medium.

Table I. *Long-chain fatty-acyl CoA and free CoA contents in the spontaneously beating whole atria at the end of the hypoxic incubation.*
Values are the average of 7 atria ± S.E.M, expressed as nmoles/g wet weight. Exposure to 20 mM L-carnitine began 60 min before the onset of hypoxia.

	Long-chain fatty-acyl CoA		Free CoA	
	Normal medium	Medium with Carnitine	Normal Medium	Medium with Carnitine
Fed rats	25.4 ± 6.0*	31.8 ± 6.0*	37.4 ± 4.0*	36.4 ± 2.7
24-h fasted rats	86.8 ± 17.4	95.0 ± 18.8	23.8 ± 4.0	30.2 ± 1.9

* $p < 0.05$ versus the fasted rat atria in the same medium.

Table II. *Lactate output during the hypoxic incubation.*
Values are means ± S.E.M., expressed as mmoles/100 mg wet weight. Between brackets the number of atria. Exposure to L-carnitine began 60 min before the onset of hypoxia.

	Paced left atria		Spontaneously beating whole atria	
	Normal medium	Medium with Carnitine	Normal Medium	Medium with Carnitine
Fed rats	15.3 ± 2.2 (8)	15.2 ± 2.5 (8)	7.3 ± 0.4 (7)	7.3 ± 0.4 (7)
24-h fasted rats	13.2 ± 1.7 (5)	11.6 ± 1.3 (5)	7.2 ± 0.9 (7)	8.3 ± 0.7 (7)

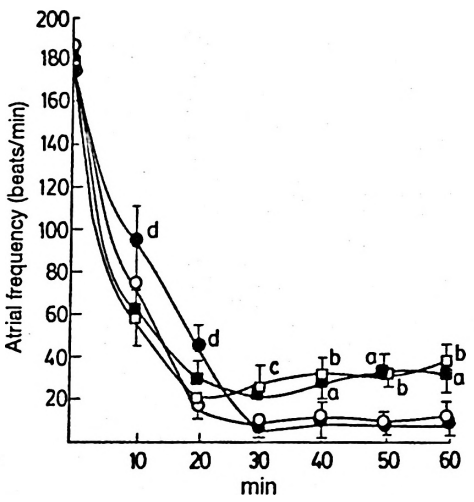

Fig. 3. *Changes of the atrial rate during the hypoxic incubation.*
Symbols as in fig. 1. Values are the average of 8 atria ± S.E.M. a: $p < 0.01$ vs the fasted rat atria in the normal medium. b: $p = 0.01$ vs the fasted rats in the carnitine containing medium. c: $p < 0.05$ vs the fasted rat atria in the carnitine containing medium. d: $p < 0.01$ vs the fasted rat atria in the carnitine containing medium.

as free CoA was reduced. These findings reflect the fast oxidation rate of fatty acid derived from the endogenous triacylglycerol that occurs in the fasting state. Upon the addition of 20 mM carnitine the levels of both metabolites remained unchanged. During hypoxia, lactate was released into the medium either in the spontaneously beating atria and the paced left atria (table II). Carnitine 20 mM did not change the glycolytic activity in all the experimental groups.

Discussion

Carnitine did not affect the fall of the peak tension during hypoxia nor during the post-hypoxic recovery, either in the fed or fasted rats atria, even though the fatty acid catabolism is increased in the fasted animals. Furthermore, carnitine worsened the contracture development and the decline of the pacemaker activity.

These findings, at least partly, lend support to several investigations wherein carnitine did not ameliorate the hypoxic and ischemic disturbances (16, 21, 24). They also coincide with the detrimental effects of carnitine described by HEARSE *et al.* (7) in the ischemic rat heart. However, in other studies carnitine showed to be beneficial to the hypoxic and ischemic hearts, although these effects were usually attained after brief (1 - 20 mM) treatment periods. Since carnitine uptake is very slow (1, 35), these prompt responses may be the reflection of properties of carnitine quite removed from effects on fatty acid metabolism. Most likely, they could be the consequences of the effects of carnitine on coronary circulation (24, 30), inotropic activity (2, 30) and membrane excitability (5, 25). However, the ineffectiveness of carnitine on peak developed tension here reported, do not seem due to its slow uptake. It was previously demonstrated that treatments longer than 30 min using concentrations similar to those presently tested, increase the intracellular carnitine levels (1, 35). On the other hand, the adverse effects of carnitine could be an untoward side-reaction to the high concentrations used, unlinked to its metabolic role. However, this explanation seems unlikely because at the end of the 60 min exposure to carnitine before the onset of hypoxia, functional parameters of control and treated atria were similar. Obviously, the mechanisms underlying the detrimental effects of carnitine cannot be explained on the bases of present data.

In accordance with the lack of beneficial effects, the atrial stores of long-chain fatty-acyl CoA and free CoA were not changed by carnitine. These findings agree with previous observations in the hypoxic rat heart (28) whereas in the ischemic hearts it has been reported that carnitine could be either ineffective (14, 31) or able to lower the acyl-CoA content (13). In addition, carnitine failed to change the lactate output. In contrast, BRODERICK *et al.* (1) reported that carnitine increased the glycolytic flux and glucose oxidation, although their findings may not be comparable to ours because they were obtained in oxygenated hearts.

In summary, from present data concerning metabolic and functional parameters, it may be concluded that carnitine does not seem beneficial to the rat atria under oxygen-limited conditions.

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A. VARELA, V. DALAMON, S. SACKS, M. CARREGAL y E. A. SAVINO. *Efecto de la L-carnitina sobre las aurículas hipóxicas de rata.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (4), 201-206, 1995.

Se determina si la L-carnitina, cofactor esencial para la transferencia a la mitocondria de ácidos grasos, alivia las alteraciones producidas por la hipoxia en la aurícula aislada de rata. Durante la hipoxia, la aurícula libera lactato, desarrolla contractura y sufre una disminución de la actividad marcapaso y de la fuerza pico. La aurícula de ratas en ayunas muestra una exacerbación de las perturbaciones funcionales durante la hipoxia y una menor recuperación al reoxigenar. Al final del período de hipoxia, las aurículas de rata ayunadas tienen un menor contenido de CoA libre y triplican su contenido de ésteres de CoA con resíduos acilo de cadena larga. El agregado de L-carnitina 5 mM, 60 min antes de iniciar la hipoxia, no muestra ningún efecto. En cambio, la L-carnitina 20 mM acelera la disminución de la frecuencia de las aurículas de ratas en ayunas y empeora la contractura en ambos estados de nutrición. La caída de la fuerza pico y la recuperación al reoxigenar, los contenidos de CoA libre y de ésteres de CoA de cadena larga y la liberación de lactato no se modifican con el tratamiento con L-carnitina 20 mM. Los resul-

tados sugieren que la L-carnitina no es beneficiosa para las aurículas hipóxicas, incluso cuando se aumenta el catabolismo de los ácidos grasos mediante el ayuno previo.

Palabras clave: Carnitina, Hipoxia, Ayuno, Aurícula de rata.

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