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Acute administration of carnitine to rats modified the basal and A23187-stimulated release of eicosanoids from 4 day carrageenin-elicited peritoneal macrophages

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Abstract

Carnitine preferentially stimulates the basal and calcium-ionophore (A23187)-stimulated synthesis and release of 6-keto-$PGF_{1\alpha}$ from carrageenin-elicited peritoneal macrophages *ex-vivo*.

Introduction

L-carnitine (β-hydroxy-(-N-trimethylammonio)-butyrate) is a natural amino acid which is essential for the transport of long chain fatty acids into mitochondria for β-oxidation [1]. Recently carnitine has been shown to enhance the formation of arachidonic acid (AA) from linoleic acid by isolated hepatocytes and to inhibit human neutrophil superoxide production [2, 3]. As cyclooxygenase (CO) and lipoxygenase (LO) metabolites of AA (eicosanoids) are important modulators of macrophage functions, including superoxide production, [4] we investigated the possibility that L-carnitine could also modulate macrophage eicosanoid synthesis.

Materials and methods

Male Wistar rats (16 weeks, four rats/group) were given 300 mg carnitine/kg dissolved in 1 ml distilled water, or distilled water alone, by intubation on days 1–4. On day 1 all animals were injected with 2 ml of a carrageenin solution (1 mg/ml) intraperitoneally. On day 4, 1 h after the last administration of carnitine, the macrophages were isolated from pooled peritoneal washes by density gradient centrifugation and suspended at 2×10^6 nucleated cells/ml in Dulbecco's modified Eagle's medium. Portions (1 ml) of the macrophage preparation were incubated at 37 °C for either 2 h (basal release) or for 30 min with 10^{-7} *M* A23187 (ionophore-stimulated release). The cells were then centrifuged and the supernatant fractions analysed for thromboxane (TX) A_2 (analysed as TXB_2) prostaglandin (PG) E_2, prostacyclin (PGI_2) (analysed as 6 keto-$PGF_{1\alpha}$) and leukotriene (LT) B_4 by radioimmunoassay.
In other experiments carrageenin-elicited macrophages from control rats were incubated with carnitine for 2 h or 24 h. The supernatant fractions were also analysed for eicosanoids.

Results

Carnitine significantly ($p<0.005$) reduced the number of macrophages isolated 4 d after an intraperitoneal injection of carrageenin: control 40 ± 11, carnitine 19 ± 6 (Values are mean $\pm$ SD; $\times 10^6$; $n=3$).
The basal synthesis and release of PGE_2, 6 keto-$PGF_{1\alpha}$ and LTB_4 was stimulated by carnitine treatment. In contrast, TXB_2 formation was inhibited (Table 1). As a result the 6 keto-$PGF_{1\alpha}$: TXB_2 ratio increased (control ratio 0.22; carnitine ratio 0.86). A23187 stimulated the release of the four ei-

Table 1
The effect of carnitine on the basal and A23187-stimulated release of eicosanoids from carrageenin elicited rat peritoneal macrophages. Eicosanoid synthesis and release (ng/2 × 10^6 nucleated cells)

Eicosanoid	Control		A23187(10^{-8} *M*)	
	Control	Carnitine	Control	Carnitine
TXB_2	5.09	2.86*	7.28	8.13
6-keto-$PGF_{1\alpha}$	1.19	2.26*	4.95	22.26*
PGE_2	1.05	1.50*	3.75	3.58
LTB_4	0.065	0.104*	0.838	1.644*

Mean values were significantly different from the corresponding control values (Mann-Whitney U-test): $*p<0.05$.

cosanoids assayed. Synthesis of 6 keto-$PGF_{1\alpha}$ and LTB_4 was further enhanced by carnitine. There was no effect on PGE_2 or TXB_2 formation. In the presence of A23187 there was a preferential increase in 6 keto-$PGF_{1\alpha}$ synthesis, relative to both TXB_2 (control ratio 1.02; carnitine ratio 4.95) and LTB_4, (A23187 control ratio 6.70; A23187+ carnitine 15.19) (Table 1).
Carnitine had no effect on macrophage eicosanoid release during incubations of 2 h or 24 h, (values not shown).

Discussion

The results presented here clearly show that the acute oral administration of carnitine modified the ability of macrophages to synthesize eicosanoids. It is not clear what mechanisms are involved. No changes in LDH or eicosanoid release were detected after incubating macrophages with carnitine *in vitro* (data not presented) so that it would appear unlikely that carnitine acted indirectly by damaging the macrophages or directly by stimulating phospholipase, CO or LO activities. It is possible that carnitine stimulated AA formation [2] but that we did not detect this *in vitro* because we incubated our macrophages in a serum free medium. Of particular interest is the finding that carnitine treatment increased the 6 keto-$PGF_{1\alpha}$:TXB_2 ratio, (basal and A23187-stimulated) and 6 keto-$PGF_{1\alpha}$:LTB_4 ratio (A23187-stimulated). A decrease in the PGI_2:TXA_2 ratio is thought to be an important factor in the progression of some cardiovascular diseases [5] while the stimulatory effect of LTs are only maximally observed in the absence of CO products [6]. Thus, changes in eicosanoid ratios induced by feeding carnitine could have important implications for regulation of inflammatory events in general. This concept is supported by the finding that cell infiltration into the peritoneum, in response to chemotactic stimuli induced by carrageenin, was reduced by carnitine treatment. This fact, together with the report that carnitine inhibits neutrophil superoxide production [3], suggests that carnitine could be anti-inflammatory *in vivo*.

Acknowledgements

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