**Introduction**

Fatty acids are oxidized in mitochondria. They are activated before they enter the mitochondrial matrix. Adenosine triphosphate (ATP) drives the formation of a thioester linkage between the carboxyl group of a fatty acid and sulphhydryl group of coenzyme A (CoA). This reaction occurs on the outer mitochondrial membrane, where it is catalysed by acyl CoA synthetase (also called fatty acid thiokinase). The activation of a fatty acid occurs in two steps. First, the fatty acid reacts with ATP to form an acyl adenylate. In this mixed anhydride, the carbonyl group of a fatty acid is bonded to the phosphoryl group of adenosine triphosphate (AMP). The sulphhydryl group of CoA then attacks the acyl adenylate, which is tightly bound to the enzyme, to form acyl CoA and AMP.

Fatty acids are activated on the outer mitochondrial membrane, whereas they are oxidized in the mitochondrial matrix (Fig. 1). Long-chain acyl CoA molecules do not readily transverse the inner mitochondrial membrane, and so a special transport mechanism is needed. Activated long-chain fatty acids are carried across the inner mitochondrial membrane by l-carnitine (β-hydroxy-(N-trimethylammonio)-butyrate), a zwitterionic compound formed from lysine. The acyl group is transferred from the sulphur atom of CoA to the hydroxyl group of carnitine to form acyl carnitine. This reaction is catalysed by carnitine acyltransferase I, which is located on the cytosolic face of the inner mitochondrial membrane. Acyl carnitine is then shuttled across the inner mitochondrial membrane by a translocase. The acyl group is transferred back to CoA on the matrix side of the membrane. This reaction is catalysed by acyltransferase II.1-3

CoA also has a role in the formation and breakdown of products from both the cyclooxygenase and lipooxygenase pathways of the fatty
FIG. 1. Schematic representation of the activation of long-chain fatty acids in the cytosol, the entry of acyl carnitine into the mitochondrial matrix mediated by a translocase and the oxidation of acyl CoA in eicosanoids in the mitochondrial matrix.
inner membrane. Inhibition of the mitochondrial oxidation of long-chain fatty acids during fasting causes heart or liver failure. Patients with cardiomyopathy due to carnitine loss are improved by carnitine supplementation.13-15

In the present study on the role of carnitine in the eicosanoid and cytokine production at inflammatory sites, the eicosanoids formed from basal and A23187 stimulated, and TNFα formed from basal and LPS stimulated carrageenin induced peritoneal cells of rats after feeding L-carnitine, acetyl L-carnitine (formed during β-oxidation of even-chain fatty acids) and propionyl L-carnitine (formed during β-oxidation of uneven-chain fatty acids) were determined.

**Materials and Methods**

*Animals and treatment:* Male Wistar rats were given 300 mg/kg carnitine or carnitine equivalent (acetyl carnitine and propionyl carnitine) (gifts of Sigma-Tau, Italy) dissolved in 1 ml distilled water, by intubation on days 1-4. Control animals were given distilled water. All animals were injected with 2 ml of a carrageenin (Marine Colloids Inc., USA) solution (1 mg/ml) intraperitoneally on day 1.

*Isolation/incubation of peritoneal cells:* Two series of experiments were carried out. In the first series (with neutrophil contamination >10%) on day 4, 1 h after the last administration of carnitine(s), the cells were isolated from pooled Gey’s balanced salt solution washes of the peritonea of the rats (four rats/group) by density gradient centrifugation over Lymphoprep (Nyegaard Diagnostica, Norway) and suspended in Dulbecco’s modification of Eagle’s medium (DMEM) (2 x 10⁶ nucleated cells). The harvested peritoneal cell population consisted of less than 90% macrophages and >10% PMN leucocytes. Portions (1 ml) of suspension were incubated at 37°C for either 2 h (basal release) or 30 min with 10⁻⁶ M A23187 (ionophore stimulated release). The cells were then centrifugated and the supernatant fractions analysed for production of leukotriene B₄ (LTB₄), prostaglandin E₂ (PGE₂), thromboxane B₂ (TxB₂) and 6-keto-prostaglandin F₁α (6-keto-PGF₁α) by radioimmunoassays.16,17

In the second series of experiments (without neutrophil contamination) on day 4, 1 h after the last administration of carnitine(s), the cells were obtained from each separate rat (nine rats/group) by washing the peritoneal cavity with 2 x 20 ml of phosphate buffered saline (PBS) (Oxoid, UK). The macrophages were isolated by density gradient centrifugation with Lymphoprep (Nycomed, Norway) and suspended in Dulbecco’s modification of Eagle’s medium (DMEM) (Life Technologies Ltd, UK) (2 x 10⁶ macrophages/ml). The harvested cell population consisted of >95% macrophages, approximately 3% PMN leucocytes and 2% other cells (lymphocytes, erythrocytes). Portions (1 ml) of suspension were incubated at 37°C for 30 min with or without 10⁻⁶ M A23187 (Sigma, USA) (ionophore stimulated release) and 24 h with or without 5 µg/ml LPS. The cells were then centrifugated and the supernatant fractions analysed for production of LTB₄, PGE₂, TxB₂ and 6-keto-PGF₁α by radioimmunoassays (basal and A23187 stimulated), and TNFα by MTT-tetrazolium bioassay (24 h with or without LPS).17,18

**Statistical analysis:** In the first series of experiments the values are given as the mean for each point ± SEM of three experiments. In the second series, the results are expressed as the mean ± SEM of nine experiments. Statistical significance was calculated using the two-tailed Mann-Whitney U test.

**Results**

*Effect of feeding of carnitine or its congeners on the number of carrageenin induced peritoneal cells:* In the first series (with neutrophil contamination) of experiments all three compounds significantly reduced, by about half, the number of nucleated cells isolated from peritonea 4 d after an intraperitoneal injection of carrageenin (x10⁶ ± SEM per rat): control 13 ± 2, carnitine 6 ± 1; acetyl carnitine 5 ± 1, propionyl carnitine 6 ± 0.6.16

In the second series (without neutrophil contamination) of experiments none of the compounds fed to the rats caused a decrease in the number of macrophages accumulated in the peritoneal cavity (x10⁶ ± SEM per rat): control, 3.6 ± 0.5, carnitine, 4.4 ± 0.6, acetyl carnitine, 3.3 ± 0.4, propionyl carnitine, 4.6 ± 0.4.

*Effect of feeding of carnitine or its congeners on the basal and A23187 stimulated release of eicosanoids from carrageenin induced peritoneal cells:* In the first series (with neutrophil contamination) of experiments the basal release of PGE₂, 6-keto-PGF₁α and LTB₄ was stimulated by all treatments. In contrast, TxB₂ production was inhibited by feeding carnitine and acetyl carnitine or not modified by feeding propionyl carnitine (Fig. 2, Table 1). A23187 stimulated synthesis of 6-keto-PGF₁α and LTB₄ was further enhanced by all three compounds and acetyl carnitine and propionyl carnitine treatments increased the formation of TxB₂. However, no effects on PGE₂ formation were detected (Fig. 3, Table 1). The 6-keto-PGF₁α/TxB₂ ratio, calculated from the basal and A23187 stimulated values, was increased by carnitine treatment (Table 2). In the presence of A23187 there was also an increase in the 6-keto-PGF₁α/LTB₄ ratio (Table 2).16 In the second
FIG. 2. Effect of feeding of carnitine or its congeners on the basal release of prostaglandin E\(_2\), thromboxane B\(_2\) and 6-keto-prostaglandin F\(_{1\alpha}\) from carrageenin induced peritoneal cells with or without neutrophil contamination. Statistical significance to the control group is shown as *p < 0.05 according to Mann-Whitney U test.

FIG. 3. Effect of feeding of carnitine or its congeners on the A23187 stimulated release of prostaglandin E\(_2\), thromboxane B\(_2\) and 6-keto-prostaglandin F\(_{1\alpha}\) from carrageenin induced peritoneal cells with or without neutrophil contamination. Statistical significance to the control group is shown as *p < 0.05 according to Mann-Whitney U test.

Discussion

It was recently shown that in a peritoneal cell population obtained from patients with ascites consisting of 77% macrophages, 16% PMN leucocytes and 7% other cells (lymphocytes, eosinophils) a marked production of 6-keto-PGF\(_{1\alpha}\) and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) took place, whereas hardly any metabolites of the cyclooxygenase pathway, but mainly products of lipoxigenase, such as LTB\(_4\) and 5-hydroxy-eicosatetraenoic acid (5-HETE) were observable with a highly purified peritoneal macrophage population (Fig. 4).\(^{19,20}\) It is thus conceivable that the increased 6-keto-PGF\(_{1\alpha}\):TxB\(_2\) and 6-keto-PGF\(_{1\alpha}\):LTB\(_4\) ratios in the first series of experiments reflected the effect of carnitine and/or its congeners on PMN leucocytes rather than on macrophages.

L-carnitine was shown in vitro to exert an inhibitory influence on chemiluminescence in phorbol-myristate-acetate stimulated human PMN leucocytes.\(^{12}\) Chemiluminescence is under the inhibitory control of those prostaglandins which exert their effect through enhanced levels of cyclic AMP via activation of the adenylate cyclase.

| Table 1. Effect of feeding of carnitine or its congeners on the basal and A23187 stimulated release of LTB\(_4\) (ng/2 x 10\(^6\) macrophages) from peritoneal cells. Values are means ± SEM |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Treatment                    | Control Carnitine | Acetyl carnitine | Propionyl carnitine |
| Neutrophil contamination Basal | 0.07 ± 0.01 0.10 ± 0.03* | 0.14 ± 0.06* | 0.12 ± 0.04* |
| + A23187                      | 0.84 ± 0.24 1.64 ± 0.38* | 1.86 ± 0.32* | 1.31 ± 0.31* |
| Without neutrophil contamination Basal | 0.41 ± 0.06 0.36 ± 0.04 | 0.30 ± 0.03 | 0.30 ± 0.03 |
| + A23187                      | 3.77 ± 1.11 1.86 ± 0.30 | 3.49 ± 1.12 | 2.67 ± 0.58 |
Effect of carnitine on eicosanoid discharge

Table 2. Effect of feeding of carnitine or its congeners on the 6-keto-PGF\textsubscript{1\alpha}-TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha}-LT\textsubscript{B}\textsubscript{4} ratios of the basal and A23187 stimulated release of the eicosanoids from peritoneal cells. Values are means ± SEM.

| Treatment                                      | Control | Carnitine   | Acetyl carnitine | Propionyl carnitine |
|------------------------------------------------|---------|-------------|------------------|---------------------|
| **Neutrophil contamination**                   |         |             |                  |                     |
| 6kPGF\textsubscript{1\alpha}-TXB\textsubscript{2} |         |             |                  |                     |
| Basal                                          | 0.22 ± 0.01 | 0.86 ± 0.12* | 1.57 ± 0.22*     | 1.00 ± 0.36*        |
| +A23187                                       | 1.02 ± 0.21 | 4.95 ± 2.33* | 1.36 ± 0.23      | 0.82 ± 0.15         |
| 6kPGF\textsubscript{1\alpha}-LT\textsubscript{B}\textsubscript{4} |         |             |                  |                     |
| Basal                                          | 21.35 ± 2.29 | 24.70 ± 7.21 | 45.40 ± 25.43   | 24.40 ± 6.09        |
| +A23187                                       | 6.70 ± 1.65 | 15.19 ± 4.10* | 10.78 ± 2.93   | 9.14 ± 2.22         |
| **Without neutrophil contamination**           |         |             |                  |                     |
| 6kPGF\textsubscript{1\alpha}-TXB\textsubscript{2} |         |             |                  |                     |
| Basal                                          | 0.48 ± 0.06 | 0.31 ± 0.03* | 0.38 ± 0.06      | 0.62 ± 0.09         |
| +A23187                                       | 0.90 ± 0.23 | 0.22 ± 0.04* | 0.42 ± 0.13      | 0.33 ± 0.04*        |
| 6kPGF\textsubscript{1\alpha}-LT\textsubscript{B}\textsubscript{4} |         |             |                  |                     |
| Basal                                          | 8.47 ± 1.14 | 5.76 ± 0.56  | 7.62 ± 0.96      | 11.41 ± 1.82        |
| +A23187                                       | 6.03 ± 1.42 | 2.18 ± 0.44* | 4.06 ± 0.86      | 3.38 ± 0.61         |

complex. Macrophages obtained from renal patients on continuous ambulatory peritoneal dialysis (CAPD) during an episode of infectious dialysis show a decrease in cyclic AMP and PGE\textsubscript{2} production and an increase in TNFα and interleukin 1β (IL-1β). A cyclic nucleotide mediated influence of PGE\textsubscript{2} is recognized in the regulation of the production of TNFα from macrophages.\textsuperscript{21,22} TNFα synthesis in peritoneal rat macrophages is up-regulated by cGMP and down-regulated by cAMP, which indicates that cyclic nucleotides act as intracellular messengers for extracellular signals of macrophage activation. Whether increased production of prostaglandins and subsequently elevated levels of cyclic AMP are involved in the effect of l-carnitine on PMN leucocytes still needs to be examined. Enhanced production of prostaglandins in PMN leucocytes might have, via an interaction with concomitantly present macrophages, implications for a conceivable influence of l-carnitine or its congeners on the release of TNFα. However we were unable to observe that TNFα formation was significantly influenced by carnitine and its congeners both in rested and stimulated macrophages (data not shown). From these negative findings one could conclude that (i) the treatment regime was inappropriate to influence the release of mediators of inflammation; (ii) in this type of inflammation a clear interrelationship between PGE\textsubscript{2} and TNFα does not exist; and (iii) the in vitro effects of carnitine and its congeners on macrophages are not representative for the in vivo situation, in which the involvement of polymorphonuclear cells and their production of prostaglandins might be of importance for the discharge of TNFα from macrophages.

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