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Early events of the exogenously provided L-Carnitine in murine macrophages, T- and B-lymphocytes: modulation of prostaglandin E1 and E2 production in response to arachidonic acid

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Abstract

L-carnitine is an essential energy-providing compound to the cell since it transports long chain fatty acids through the mitochondrial membrane and delivers them to the β-oxidation pathway for catabolism and/or entrance to biosynthetic pathways. Some of the early events taking place in immune cells after L-carnitine inoculation *in vitro* are defined in this report. Using arachidonic acid as a fatty acid source, we determined the utilization rate of L-carnitine by murine T-, B-lymphocytes and macrophages within two hours of cell culture, its effect on prostaglandin E1 and E2 production and the levels of β-hydroxy-butyrate. The results show that although all immune cells consume a small portion of L-carnitine, β-hydroxy-butyrate decreases upon addition of arachidonic acid and/or L-carnitine indicating that active biosynthetic pathways are induced. L-carnitine is shown to increase the arachidonic acid-induced production of prostaglandins E1 and E2 in macrophages, while their secretion from T- and B-lymphocytes is decreased. These findings indicate the L-carnitine may very rapidly alter the activation state of immune cells and lead to the development of various reactions, beneficial or not to the organism. © 2003 Elsevier Inc. All rights reserved.

Keywords: L-carnitine; Arachidonic acid; Prostaglandin E1 and E2; β-OH butyrate; Macrophages; T and B lymphocytes

1. Introduction

Fatty acids are catabolized *via* the β-oxidation in the mitochondrial matrix. The passage of long chain fatty acids through the mitochondrial membrane requires their coupling to coenzyme-SH and production of the corresponding thiol-ester. Under this configuration, acyl-CoA crosses the mitochondrial membrane following the L-carnitine (L-Cn) transporter system. The acyl- group is transferred to the hydroxy group of L-Cn to form the acyl-carnitine in the intermembrane space, reaction that is catalyzed by the carnitine acyl-transferase I. Acyl-carnitine is transported through the inner mitochondrial membrane by a carnitine acyl-translocase. The last step for the entrance of fatty acids in the mitochondria consists in the transfer of the acyl-group to coenzyme A (CoA), a reaction catalyzed by carnitine-acyl transferase II. Finally, L-Cn returns to the mitochondrial intramembrane space aided by the translocase in exchange with acyl-carnitine [1]. The acyl residue is then transferred to the SH group of CoA to give acyl-CoA thioester, which serves as a substrate to β-oxidation or for triglyceride biosynthesis [1]. The overall sequence of reactions during β-oxidation is the elimination of a two-carbon unit as acetyl-CoA from the acyl chain. In some cases, like in the liver for example, a portion of the semi-final product of fatty acid β-oxidation, the acetoacetyl-CoA, is used for the formation of cholesterol and β-OH-butyrate (ketone bodies) supplying energy as shown for brain and muscle cells [1].

Any damage in the transporter system such as carnitine, transferase or translocase deficiency, does not allow oxidation of long chain fatty acids. Indeed, many cases of primary and secondary carnitine deficiencies have been described:

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Primary carnitine deficiencies most commonly include myopathies and systemically progressive cardiomyopathies [2-4], whereas secondary carnitine deficiencies refer to numerous genetic and acquired conditions associated to a depletion of tissue carnitine stores. The most frequently reported secondary deficiencies are the organic acidurias [5] and the genetic defects of β-oxidation such as deficiency in short-, medium- and long-chain acyl-CoA dehydrogenases [6].

The administration of L-Cn has been shown beneficial in most of the above cases and because this compound is essential to long-chain fatty acid catabolism, many nutrients, including infantile milk, are supplemented with this agent. It has also been shown that L-Cn administration to mice affects the humoral and cellular immune responses by decreasing interleukin-2 production in response to specific antigenic stimulus [7].

In the present study we inquired whether immune cells *in vitro* can directly use L-Cn and, if so, whether it can interfere with fatty acid degradation or synthesis. We have chosen an experimental system where immune cells were cultured in a fatty acid-free medium in the presence or not of L-Cn and arachidonic acid (AA). Arachidonic acid was used as a fatty acid source since a) it is an essential ecosanoid taken up with food and endogenous production cannot interfere with our experiments and b) it generates prostaglandins (PGs), prostacyclins or thromboxane through the pathway of cyclooxygenase or leucotriens through the pathway of lipooxygenase [8].

Prostaglandins are fatty acid derivatives that resemble hormones found in all mammalian tissues. They belong to the family of bioreactive lipids produced upon highly regulated action of enzymes including cyclooxygenase 1 and 2 as well as specific prostaglandin synthases [9]. Cyclooxygenase 1 is involved in the biosynthetic pathway of thromboxane A2 in platelets, production of gastroprotective PGs, as well as regulation of salt and water uptake by kidneys. The production of cyclooxygenase 2 is favored in macrophages, fibroblasts and vascular epithelium after exposure to mechanical stress, cytokines and other growth factors and plays a key role in the biosynthetic pathway of PGs during inflammatory reactions, blood pressure regulation, reproduction etc [8].

We here concentrated on prostaglandin E1 (PGE1) and prostaglandin E2 (PGE2), which are actively involved in inflammatory as well as anti-inflammatory reactions with direct and indirect effects on the immune response, cell differentiation, nerve cell regeneration and vascular dilatation. Thus, in the present communication, employing AA as a fatty acid source, we first examined how L-Cn is utilized from the three basic types of immune cells, i.e. macrophages, T and B lymphocytes. After determining the levels of L-Cn used by the cells and their correlation to β-OH-butyrate production in the presence or not of AA, we examined whether L-Cn modulates the production of PGE1 and PGE2 from the cells. The results presented here show that, although the cells consume a small portion of L-Cn, this compound increases the AA- induced production of PGE1 and PGE2 by macrophages, while it decreases their secretion by T and B lymphocytes. In all cell types, β-OH-butyrate decreases upon addition of AA and/or L-Cn indicating that other active biosynthetic pathways are induced.

2. Materials and methods

2.1. Mice

BALB/c mice were housed in the Animal Facility of the University of Crete (Department of Biology), in rooms with controlled light cycles (12L; 12D, lights-on at 0600 hrs).

2.2. Chemicals

L-Carnitine and AA were purchased from Sigma (St Louis, MO) and used at the concentrations of 10^{-4} M (64 μg/ml) and 400 μM respectively. For PGE1 and PGE2 detection the PGE1 and PGE2 immunoassay kits (R&D Systems, MN, USA; cat No DE1200 and DE0100 respectively) were used.

2.3. Cell sorting and culture

Spleen cells from three mice each time were isolated in Hank's balanced salt solution (HBSS; Gibco BRL, Grand Island, NY) and put in single cell suspension by gently pushing the tissue through a meshwire screen. Spleen cell suspension was centrifuged at 1200 rpm for 10 min and the cell pellet resuspended in 10 ml HBSS. The cells were washed three times in HBSS and counted. Before plating, the cells were diluted in 10 ml Dulbeco's modified essential medium (DMED; Gibco BRL) which is a lipid-free medium, supplemented with 10% Fetal calf serum (FCS; (Gibco BRL) at a concentration of $1x10^6$ cells/ml and cultured in 100 mm petri dishes (Sarstedt, Numbrecht, Germany). Splenic macrophages were isolated after a 24-hr adherence. Thus, 24 hr later the non-adherent cells were discarded and the adherent cells were washed three times with DMEM. The adherent cells were scraped off the petri dish, counted and replated in 24-well plates (Sarstedt) at a concentration of $1x10^6$ cells/ml in DMEM without FCS. Arachidonic acid, with or without L-Cn, was added to the cultures for 30 min, 1 and 2 hr. Culture supernatants were thereafter collected, centrifuged and immediately assayed for PGE1 or PGE2 content using the PGE1 and PGE2 detection kits in 1:2 dilutions. The isolated cells were 99 $\pm$ 1% positive for the Mac-1 surface marker and we will be referred as macrophages thereafter.

T and B lymphocytes were isolated by negative selection cell sorting using magnetic bead particles (Dynabeads; Dynal, Oslo, Norway). Thus, spleen cells were initially plated in 100 mm petri dishes as previously described let to adhere

for 24 hr and the non-adherent spleen cells were thereafter submitted to cell sorting using anti-Ly5 or anti-Thy-1 monoclonal antibodies respectively. Non-adherent cells were incubated in parallel test tubes with anti-Ly5 or anti-Thy-1 antibodies (1μg/ml) for 30 min at 4°C and after eliminating the non-bound antibody, by washing three times with DMEM, a secondary anti-mouse IgG coupled to magnetic beads antibody was added to the cells according to the negative selection protocol of the supplier. After an incubation of 30 min at room temperature, Ly5 or Thy-1 positive cells were respectively eliminated using the Dynal magnet system. The isolation procedure was repeated three times and all cell fractions (cells that were not attached to the magnet) were pooled, centrifuged and plated at a concentration of 1x10^6 cells/ml in 24-well plates in DMEM; 10% FCS. The cells were let to rest for 5-6 hr and 18 hr prior to the addition of AA the culture medium was replaced with fresh DMEM without FCS. Arachidonic acid with or without L-Cn was added to the cultures for 30 min, 1 and 2 hr. Culture supernatants were then collected, centrifuged and immediately processed for PGE1 and PGE2 detection in 1:2 dilutions. The negatively selected cells using the anti-Ly5 or anti-Thy-1 monoclonal antibodies were stained in immunofluorescence experiments [7] by anti-Thy-1 or anti-Ly5 respectively and 100% of the cells were shown positive for the two markers, respectively.

2.4. Measurement of acyl- and free L-Cn in cell lysates and culture supernatants

L-Carnitine was measured in the supernatant of the T-, B- lymphocytes and macrophages according to the method of Mc Garry and Foster [10] as modified by Bohles et al. [11] The total carnitine assay is a measurement of free carnitine following alkaline hydrolysis, which releases additional free carnitine from the esterified forms. The amount finally of acyl-carnitine is calculated from the difference between the amounts of free and total carnitine. The percent of exogenous L-Cn used by the cells was each time calculated from the exogenously added L-Cn, 64 μg/ml and the total L-Cn detected after inoculation to the cells minus the total endogenous L-Cn: (total L-Cn –endogenous L-Cn)/64 μg/ml x 100

2.5. Detection of PGE1 and PGE2 levels in culture supernatants

The experiments were performed following the supplier's recommendations. Briefly, the assay was based on the competitive binding technique in which PGE1 or PGE2 present in the sample competes with a fixed amount of alkaline phosphatase- labeled PGE1 or PGE2 for sites on a sheep polyclonal antibody. During the incubation, the polyclonal antibody binds to the donkey anti-sheep antibody coated onto the microplate. Following wash to remove excess conjugate and unbound sample, a substrate solution is added to the wells to determine the bound enzyme activity. Immediately, following color development, the absorbance is read at 405 nm. The intensity color is inversely proportional to the concentration of PGE1 or PGE2 in the sample. All samples were run in triplicate and mean ± SEM always calculated. The results were calculated according to the standard curve that was always being generated after subtracting the non-specific binding values and the curve f(B/B0%) (binding/maximum binding) = [PGE1] or [PGE2] (pg/ml) was plotted.

3. Results

Since various studies attribute an immunomodulatory role to L-Cn, in the present report we evaluated how immune cells utilize L-Cn and concentrated on the early events taking place after inoculation of this reagent to cultures. Thus, we determined the amount of L-Cn used by splenic T-, B-lymphocytes or macrophages after 2 h of culture in the presence or not of AA and whether this promotes fatty acid degradation (by measuring β-OH butyrate) or biosynthesis (production of PGE1 and PGE2).

3.1. T lymphocytes

In the absence of any stimulus, the total amount of L-Cn in 1x10^6 T cells was calculated to be 4.6 μg/ml, where 2.9 μg/ml were detected in the cell lysates and 1.7 μg/ml in the cell supernatants. Most of the L-Cn found in the cell lysates were in a free form (2.2 μg/ml versus 0.7 μg/ml free- and acyl-L-Cn respectively) whereas culture supernatants contained exclusively acyl-L-Cn (Table 1).

In cases where L-Cn was added to the cells, although the cultures were seeded with 64 μg/ml L-Cn, only 25 μg/ml could be detected after 2 hrs of culture (corresponding to 32% of the provided quantity as calculated from the exogenously added L-Cn, 64 μg/ml and the total L-Cn detected after inoculation to the cells minus the total endogenous L-Cn, 4.6 μg/ml) and most of it was found in the culture supernatants (19 μg/ml versus 6 μg/ml found in the cell lysates). L-carnitine detected in the culture supernatants was found to be exclusively in the free form whereas 3.7 and 2.3 μg/ml acyl-L-Cn and free L-Cn were detected in the cell lysates respectively (Table 1).

The presence of AA to the cells did not considerably alter the distribution of acyl- and free-L-Cn in the culture supernatants and cell lysates as compared to untreated cells. The only remarkable difference was noted in the ratio of acyl-L-Cn over free L-Cn in the cell lysates, which increased from 0.32 in the untreated cells to 1.0 in the AA-treated cells (Table 1).

In the presence of both AA and L-Cn the total amount of L-Cn increased to 41 μg/ml, most of which (34 μg/ml) was still found in the culture supernatants in a free form. In the

Table 1

	T Cells		
	L-Cn (μg/ml)		
		Cells	Acyl Free
Treatment[a]	Total/ 10^6 cells	Supernatants	Acyl Free
Control	4.6	2.9	0.7 2.2
		1.7	1.7 0
L-Cn	25	6	3.7 2.3
		19	0 19
AA	4.4	3	1.5 1.5
		1.4	1.4 0
L-Cn + AA	41	7	3.2 3.8
		34	0 34

[a] Cells were treated either with 64 μg/ml (10^{-4}M) L-carnitine (L-Cn) or 400 μM arachidonic acid (AA) or both for two hours.

cell lysates the ratio acyl-L-Cn/free L-Cn decreased from 1.6 in the L-Cn -treated cells to 0.84 in the AA and L-Cn-treated cells (Table 1).

The production of PGE1 and PGE2 followed a similar pattern. Thus, minimal quantities were detected in untreated cells and L-Cn-treated cells, which increased in the presence of AA by 97 and 83% for PGE1 and PGE2 respectively as compared to untreated cells (Fig. 1). The presence of AA to the L-Cn-treated cultures, however, decreased the amount of PGE1 and PGE2 by 44 and 5% respectively as compared to the cultures stimulated with AA alone (Fig. 1).

Beta-OH-butyrate, measured in order to evaluate the pathway of fatty acid degradation, was decreased in the presence of L-Cn by 50% as compared to control untreated cells and by 40% as compared to AA-treated cells indicating that L-Cn did not convert fatty acids to ketone bodies (Fig. 1).

3.2. B lymphocytes

In untreated B cells, the total amount of L-Cn was found to be 5.9 μg/ml, where 3.6 μg/ml were detected in the cell lysates and 2.3 μg/ml in the cell supernatants. In contrast to T cells, all L-Cn was found in the acyl form. As expected, exogenously added L-Cn (64 μg/ml) increased the total amount to 24 μg/ml (corresponding to 28% of the provided quantity) from which 6 μg/ml were present in the cells lysates in an acyl form and 18 μg/ml in the culture supernatants as free L-Cn (Table 2).

The presence of AA resulted in similar distribution of L-Cn as in untreated controls whereas simultaneous addition of AA and L-Cn resulted in a distribution similar to that of L-Cn-treated cells (Table 2).

Similarly to T cells, PGE1 and PGE2 were produced in minimal amounts in non-treated and L-Cn-treated groups (Fig. 2). The presence of AA increased PGE1 and PGE2 by 96 and 94% respectively as compared to untreated cells, which, upon addition of L-Cn to the AA-treated cells, decreased by 38 and 26% respectively (Fig. 2).

Beta-OH-butyrate decreased by 40% when AA was added to the untreated cells, while it increased by 33% when AA was added to the L-Cn-treated cells (Fig. 2).

3.3. Macrophages

Untreated cells contain 4.4 μg/ml total L-Cn, which was equally distributed to the cell lysates and culture supernatants and it was only found in the acyl form. In the presence of exogenously added L-Cn, 23 μg/ml of total L-Cn were recovered (29% of the provided quantity) where 5 μg/ml were detected in the cell lysates and the remaining 18 μg/ml in the culture supernatants in a free form. Within the cell lysates, 3 μg/ml corresponded to acyl-L-Cn and 2 μg/ml to free L-Cn (Table 3).

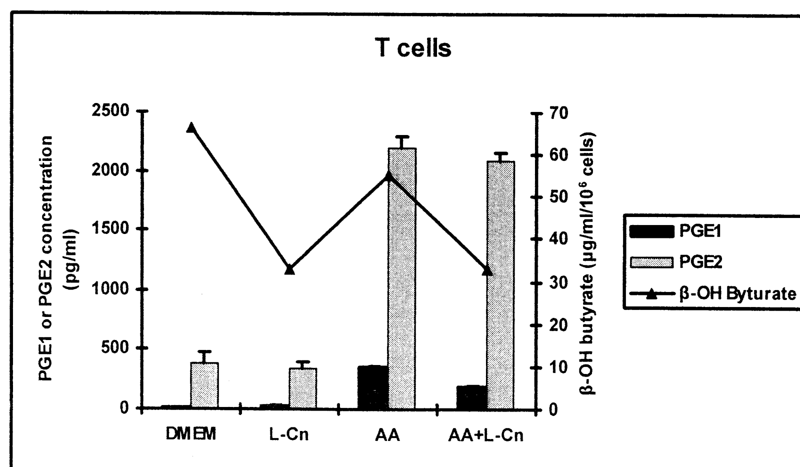

Fig. 1. Production of PGE1 or PGE2 by T cells and detection of their content in β-OH butyrate in the presence of L-carnitine (L-Cn), arachidonic acid (AA) or both. One million T cells were cultured under the above conditions for 2 hr and culture supernatants were tested for the presence of PGE1 or PGE2 (left axis) The cells were homogenized and tested for their content in β-OH butyrate (right y axis) as described in the section of Methods.

Table 2

B Cells				
	L-Cn (μg/ml)			
		Cells	Acyl	Free
Treatment[a]	Total/ 10^6 cells	Supernatants	Acyl	Free
Control	5.9	3.6	3.6	0
		2.3	2.3	0
L-Cn	24	6	6	0
		18	0	18
AA	5.6	3.3	3.3	0
		2.3	2.3	0
L-Cn + AA	27	5	5	0
		22	0	22

[a] Cells were treated either with 64 μg/ml (10^{-4}M) L-carnitine (L-Cn) or 400 μM arachidonic acid (AA) or both for two hours.

Table 3

Macrophages				
	L-Cn (μg/ml)			
		Cells	Acyl	Free
Treatment[a]	Total/ 10^6 cells	Supernatants	Acyl	Free
Control	4.4	2.2	2.2	0
		2.2	2.2	0
L-Cn	23	5	3	2
		18	0	18
AA	4	3	1	2
		1	1	0
L-Cn + AA	23	4	3.80	0.2
		19	0	19

[a] Cells were treated either with 64 μg/ml (10^{-4}M) L-carnitine (L-Cn) or 400 μM arachidonic acid (AA) or both for two hours.

Arachidonic acid seemed to favor accumulation of L-Cn in the cells since 75% of the total L-Cn was detected in the cell lysates, where 1/3 was found in acyl form and 2/3 corresponded to free L-Cn. The 25% of the total L-Cn detected in the culture supernatants was under the acyl-form (Table 3).

Simultaneous addition of AA and L-Cn to macrophages increased as expected total L-Cn to 23 μg/ml from which 4 μg/ml were present in the cell lysates and 19 μg/ml in the culture supernatants as free L-Cn. In the cell lysates, however, most of L-Cn was in the acyl form (3.8 μg/ml) and only a small amount (0.2 μg/ml) of free L-Cn was detected (Table 3).

As it is already known from the literature, macrophages are potent producers of PGE1 and PGE2. The results presented here showed that indeed untreated cells produce 2.8 and 1.5 ng/ml of PGE1 and PGE2 respectively (Fig. 3). These amounts slightly decreased in the presence of L-Cn, but increased to 6.7 and 2.7 ng/ml for PGE1 and PGE2 respectively in the presence of AA (Fig. 3). This increase was further pronounced when L-Cn was added to the AA-treated macrophages, reaching thus 13.5 and 3.7 ng/ml for PGE1 and PGE2 respectively (Fig. 3).

Finally, the levels of β-OH-butyrate did not considerably change when AA or AA and L-Cn were added to the cells. Only in cases when L-Cn alone was added to the macrophage culture, β-OH-butyrate showed a 4-fold decrease indicating that, in the absence of any lipid nutrient in the culture medium, L-Cn possibly drives the fatty acid reserves of the cells to other metabolic pathways (Fig. 3).

4. Discussion

L-Cn is an important L-lysine derivative that ensures the long chain fatty acid transport through the mitochondrial membrane and their delivery to the β-oxidation pathway, which, following sequential reactions, eliminates every time acetyl-CoA from the acyl chain until its complete degradation. Intermediate, however, products can enter various bio-

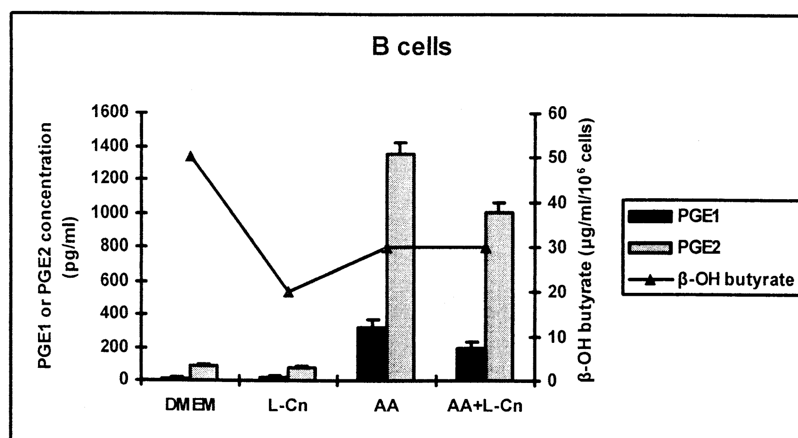

Fig. 2. Production of PGE1 or PGE2 by B cells and detection of their content in β-OH butyrate in the presence of L-carnitine (L-Cn), arachidonic acid (AA) or both. One million B cells were cultured under the above conditions for 2 hr and culture supernatants were tested for the presence of PGE1 or PGE2 (left axis) The cells were homogenized and tested for their content in β-OH butyrate (right axis) as described in the section of Methods.

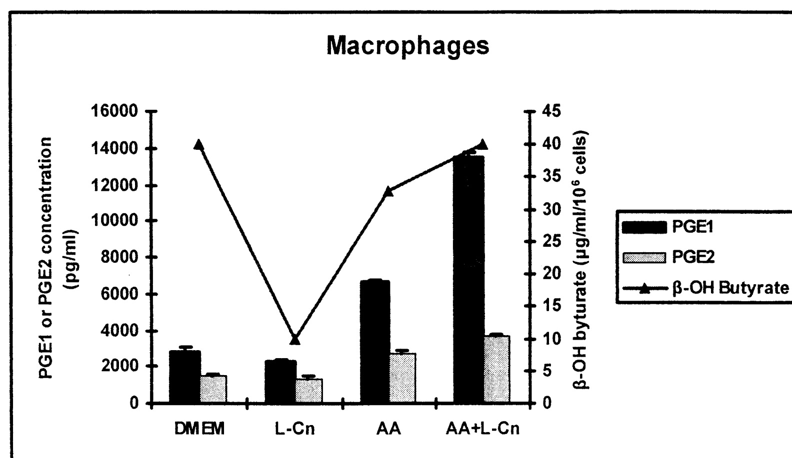

Fig. 3. Production of PGE1 or PGE2 by macrophages and detection of their content in β-OH butyrate in the presence of L-carnitine (L-Cn), arachidonic acid (AA) or both. One million of macrophages, isolated as described in the section of Methods, were cultured under the above conditions for 2 hr and culture supernatants were tested for the presence of PGE1 or PGE2 (left axis) The cells were homogenized and tested for their content in β-OH butyrate (right axis) as described in the section of Methods.

synthetic pathways and in some cases the semi-final product acetoacetyl-CoA is used for the formation of cholesterol and ketone bodies. In previous studies we have shown that *in vivo* administration of L-Cn to DNP-HSA-immunized mice results in a decrease of the number of macrophages and interleukin 2 production [7]. Furthermore, acetyl-L-Cn has been shown to increase sperm mobility [12,13], retard development of disease in Alzheimer's patients and have a positive effect on the mini mental status of the patients [14,15]. In other experimental protocols, L-Cn has been shown to increase antibody production by hybridoma cell lines, tumor necrosis factor-β, interleukin-6 and interleukin-1β production from peripheral blood cells [16] and inhibit the development of intracellular apoptotic signals [17-19].

In the present study we defined the early events taking place in immune cells upon administration of L-Cn *in vitro.* Using a fatty acid free culture system, we examined the mobilization of internal L-Cn as well as the effect of exogenously added L-Cn. To evaluate the effect of L-Cn in fatty acid catabolism by immune cells we used AA as a fatty acid source, which could provide easily measurable biosynthetic products (PGE1 and PGE2) and account for the immunosuppressive activity of L-Cn previously described. Thus, the distribution of free or acyl-L-Cn in the cell lysates and culture supernatants was determined following a short-term treatment with L-Cn and/or AA in T, B lymphocytes and macrophages in lipid free medium. This information was further correlated to PGE1 and PGE2 production to evaluate the influence of L-Cn in biosynthetic pathways and to β-OH-butyrate in the cells for estimating the involvement of L-Cn in the catabolic pathway.

Each cell type tested showed a different pattern in L-Cn mobilization and utilization. Thus, T cells in the absence of exogenously added L-Cn, regardless of the AA presence or not, exhibited only acyl-L-Cn production in the culture supernatants. In the cells lysates of untreated cells most L-Cn was found in a free form whereas in AA-treated cells equal amounts of free and acyl-L-Cn were detected. The increase of acyl-L-Cn in the cell lysates was accompanied with increase of PGE1 and PGE2 by 97 and 83% respectively and decrease of β-OH-butyrate (by 17%) indicating that some portion of AA was transferred through L-Cn to the biosynthetic pathway of PGs. Upon exogenous addition of L-Cn, only a small portion, corresponding to 32% of the provided quantity (see Methods for calculations) was used by the cells and the remaining was possibly catabolized.

In the presence of exogenous L-Cn, only the free form was detected in the culture supernatants whereas cells lysates contained both acyl and free forms showing that T cells entered an active stage in fatty acid transport and biosynthesis. This latter statement correlated with a decrease in β-OH-butyrate (50% reduction) implying that endogenous fatty acids did not go to terminal degradation but entered other biosynthetic pathways. These pathways, however, did not include PGE1 or PGE2 production. Simultaneous administration of AA and L-Cn to T cells allowed them to retain 57% of the exogenously elaborated L-Cn, yet most of it was found in the culture supernatants as free L-Cn. Within cells lysates both acyl- and free-L-Cn were detected in equivalent amounts. The addition of AA to the L-Cn-treated cells did not alter the levels of β-OH-butyrate while it decreased the production of PGE1 and PGE2.

In contrast to T cells, B lymphocytes showed a more stable pattern as far as the mobilization of L-Cn is concerned. In all cases cell lysates contained exclusively acyl-L-Cn, which increased when AA was added to the cultures. In the absence of exogenously added L-Cn, culture supernatants contained acyl-L-Cn whereas in the presence of L-Cn they contained only the free form of L-Cn. Despite the presence of AA, B cells retain only 1/3 of the exogenously added L-Cn. Arachidonic acid increased the production of

PGE1 and PGE2 by 96 and 94% respectively as compared to untreated controls, a finding correlated to a decrease in β-OH-butyrate suggesting that part of the acyl-L-Cn was driven to PGE1 and PGE2 production rather than completely catabolized. The simultaneous presence of AA and L-Cn, although did not alter the levels of β-OH-butyrate, it decreased PGE1 and PGE2 production.

Finally, macrophages resemble T cells as to L-Cn mobilization. They retain only 29% of the exogenously provided L-Cn and show a significant alteration of the acyl and free forms in the cell lysates as compared to untreated cells. Although only acyl-L-Cn is detected in untreated cells, in the presence of AA, 2/3 of L-Cn was found in the free form, which, as expected, was accompanied by an increase of PGE1 and PGE2 as well as a slight decrease in β-OH-butyrate. Exogenous L-Cn increased the acyl and the free L-Cn forms as compared to untreated controls, decreased β-OH-butyrate (by 75%) and slightly decreased PGE1 and PGE2 production (reduction by 20 and 12% respectively). Simultaneous addition of L-Cn and AA to macrophages reduced free-L-Cn in the cell lysates and increased PGE1 and PGE2 production by 50 and 27% respectively as compared to AA-treated cultures. In the culture supernatants, a similar to T- and B-cell pattern concerning the acyl and free L-Cn distribution, was obtained.

Comparing the three cell types, it can be argued that T cells, by showing an active alteration between free and acyl-L-Cn, are enforced towards cell activation. Macrophages exhibit an intermediate pattern of activation, which can be triggered by the simultaneous presence of AA and L-Cn and in such case PGE1 and PGE2 are part of their activation products. On the contrary, B cells show a stable stage of activation and none of the applied treatments could considerably change their activity.

With the exception of macrophages, the production of PGE1 and PGE2 was reduced in the presence of L-Cn in AA-treated cells showing that this pathway is not favored in B and T lymphocytes. As already mentioned, intermediate products from β-oxidation sequential reactions may be used for triglyceride formation whereas the semi-final product, aceto-acetyl coA, can be used for the synthesis of cholesterol and ketone bodies. In order to study the possibility of such alternative pathways in all three types of cells, cholesterol and triglycerides were measured (data not shown). Their absence, however, from the cell lysates as well as the culture supernatants suggests that different than the above types of reactions, not yet characterized, occur in these cells.

In summary, it is shown that in the absence of any stimulus, L-Cn is found exclusively in the acyl form in the culture supernatants of primary immune cells. The addition of AA or L-Cn to macrophages and T cells, but not B cells, mobilizes acyl-L-Cn and some free L-Cn appears. Most of the L-Cn added to the cells is found in the culture supernatants in a free form. Yet, it is unknown if this corresponds to the exogenous not used L-Cn, to endogenous release of L-Cn, or to exogenous L-Cn up taken, used and then released from the cells. It is obvious that exogenously provided L-Cn mobilizes fatty acid reserves from the immune cells and alters their energetic state, which can thereafter influence various pathways affecting cell maturation and changes in cytokine production, events that determine the necessary reactions for a balanced function of the organism.

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