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The Influence of Serum Fatty Acid Binding Proteins on Arachidonic Acid Uptake by Macrophages

Mikhail L. Strokin, Marina G. Sergeeva,* and Alevtina T. Mevkh

A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 191899, Moscow, Russia, E-mail: sergeeva@libro.genebee.msu.su

Abstract

The role of serum fatty acid binding proteins (FABPs) in arachidonic acid (AA) uptake by murine peritoneal macrophages has been studied. The kinetics of [^{3}H]arachidonic acid uptake by the cells was investigated over a wide range of AA concentration (10^{-10}–10^{-5} *M*). It was shown that these putative fatty acid transporters dramatically change the uptake processes. In the presence of FABPs, the time-course curves of AA uptake exhibited two distinct periods: one with a rapid AA uptake during the first hour with an equilibrium in 1–2.5 h and another with an equilibrium reached in 20 h, whereas in the absence of FABPs the uptake curves were smooth without kinks and with the equilibrium reached in 10 h. In addition, it was shown that the amount of incorporated AA was linearly dependent on the concentration of AA over the range of 10^{-10}–10^{-6} *M* in the presence of serum FABPs and 10^{-10}–10^{-7} *M* in their absence.

We assume that the changes in the character of AA uptake by macrophages in the presence of FABPs occur due to the interaction of FABPs with the cell plasma membrane.

Index Entries: Arachidonic acid; fatty acid metabolism; fatty acid binding proteins; macrophages.

Introduction

Arachidonic acid (AA) (5,8,11,14-eicosatetraenoic acid, 20:4[n-6]) is a polyunsaturated fatty acid known to be a precursor of a wide range of physiologically active substances, the so-called eicosanoids. These substances are formed by AA oxygenation via cyclooxygenase (prostaglandins and thromboxanes), lipoxygenase (HETEs and leukotrienes), or

*Author to whom all correspondence and reprint requests should be addressed.

epoxygenase (epoxy-eicosatrienoic acids and HETEs) pathways *(1)* and regulate such important physiological processes as thrombogenesis, inflammation, and immune reactions *(2–4)*. Furthermore, according to recent data AA itself is an active substance, in particular, AA has been shown to modulate different plasma-membrane ion channels, to mobilize Ca^{2+} from internal stores *(5)*, and to regulate gene expression at the transcription level *(6)*.

AA enters the organism through diet or is synthesized in the liver. In blood plasma the concentration of free AA is very low, since it forms complexes with serum fatty acid binding proteins (FABPs) *(7)*. These complexes are responsible for AA delivery to the cells. The first step of AA uptake by the cells is penetration through the cell membrane. Inside the cells AA could be bound by specific intracellular FABPs and transformed into active metabolites or incorporated into membrane phospholipids *(8)*. Therefore, AA binding to FABPs, uptake, and incorporation into membrane phospholipids are crucial for AA functions. A number of FABPs are involved in extracellular and intracellular transport of AA (*8*). Meanwhile, the mechanism of regulation of AA uptake and AA participation in cell signaling by serum FABPs is still unknown.

The aim of our work was to investigate the kinetics of AA uptake by murine peritoneal macrophages and the modulation of the uptake by serum FABPs.

Methods

Cell Culture

Resident mouse macrophages were obtained by peritoneal lavage of untreated F1 mice with sterile RPMI-1640 medium (ICN), containing 2 m*M* L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin. Cells were counted and plated onto 96-well plates ($1.8–2 \times 10^5$ cells/well) in 200 mL RPMI-1640 with 7.5% heat-inactivated fetal calf serum (FCS, Sigma). The viability of the cells, as judged by Trypan Blue exclusion, was never below 96%. To attach macrophages to the culture dishes, the cells were incubated for 2–2.5 h at 37°C in humidified air with 5% CO_2. Then adherent cells were double washed with PBS, and fresh RPMI-1640 was added.

Study of the Uptake Kinetics

Media with different concentrations of AA but the same radioactivity were prepared by mixing RPMI-1640 containing 1×10^{-10} *M* [^{3}H]AA (1.92×10^{-8} Ci/mL) with an appropriate amount of nonradioactive AA. FCS served as FABPs source and was added to the media to a final concentration of 2%. In all cases the AA-FABPs equilibrium was achieved within 20 s at 25°C *(9)*. After that, the media were added to the cells. The 180-µL aliquots of culture medium were taken out at fixed time intervals. Radioactivity of the samples was determined by liquid scintillation counting.

Results and Discussion

Macrophages are known to metabolize AA actively. These cells play an important role in inflammation and immune response development *(10)*. We used the murine peritoneal macrophages in our investigation. Previous studies on fatty acid interactions with cells were mostly conducted with serum albumin or FCS as a source of FABPs *(8)*. Besides albumin, FCS contains α-fetoprotein, e.g., the protein interacting with fatty acids with parameters similar to those for albumin–fatty acid interactions. We have compared the in vitro kinetics of [^{3}H]AA uptake by murine peritoneal macrophages with and without serum FABPs.

The time-course of AA uptake in the presence and in the absence of serum FABPs were different from each other (Fig. 1). The difference was dependent on AA concentration: over the range of 10^{-10}–10^{-7} *M* AA, the AA uptake level within the first hours without serum FABPs was considerably higher than in their presence and within 18–20 h the difference in uptake became insignificant, reaching 50% in both cases (Fig. 1A and B); at 10^{-6} *M* AA the initial AA uptake level was higher without FABPs, however, the final AA uptake level was significantly lower in this case and was about 32 vs 45% in the presence of FABPs (Fig. 1C); with 10^{-5} *M* AA the time-course curves were very close to each other with the final uptake level of 28–30% (Fig. 1D).

We have to emphasize that at any time of observation in the presence of FABPs, the amount of incorporated AA was linearly dependent on AA concentration over the range 10^{-10}–10^{-6} *M* AA. The time-course of AA uptake without FABPs was linearly dependent on AA concentration both over the range 10^{-10}–10^{-7} *M* and over 10^{-6}–10^{-5} *M*, i.e., saturation with AA wasn't achieved. The data can be deduced from those presented in Fig. 2.

We assume that these changes occur due to the regulation of AA metabolism by the substance itself. The putative active AA concentration is about 10^{-5} *M (11,12)*. The data presented in results of Fig. 2B show similar uptakes at 10^{-6} and 10^{-5} *M* AA in the absence of FABPs. We suppose that this phenomenon reflects the higher concentration of AA in the absence of FABPs than with them and that the concentration of free unbound AA in medium is essential for the regulatory effect to occur.

In our experiments, FCS was used as a source of FABPs. The FABPs concentration was less than 5×10^{-6} *M* with 2% FCS in the medium. With 10^{-5} *M* AA a large part of AA was unbound. Therefore, FABPs had no effect on the uptake under these conditions (Fig. 1D).

We observed two distinct periods on the time-course curves of AA uptake in the presence of FABPs and 10^{-10}–10^{-6} *M* AA (Fig. 2A). The first one corresponded to a rapid AA uptake during the first hour followed by an equilibrium at 1–2.5 h. The second period showed the next equilibrium plateau in 20 h. With 10^{-5} *M* AA the kinetics of AA uptake lost this two-step character.

The mechanism of the cell membrane crossing by fatty acids, which AA belongs to, is unclear. Some researchers assume that there is a system

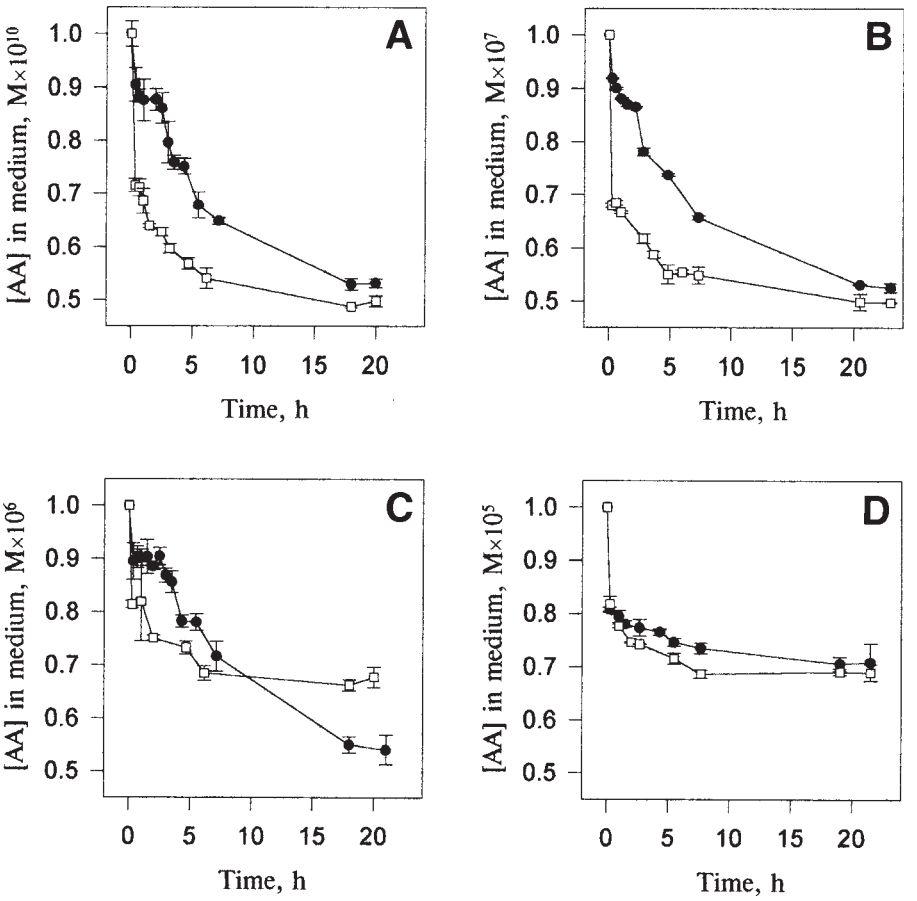

Fig. 1. AA uptake by macrophages cultivated in the medium with (●) and without (■) serum FABPs. 2% FCS were used as SFABPs source. Initial AA concentration, *M*: **(A)** 10^{-10}; **(B)** 10^{-7}; **(C)** 10^{-6}; **(D)** 10^{-5}.

of active transport of fatty acids *(13)*, others suggest that it is a simple diffusion process *(14)*.

The uptake of AA by macrophages can be described by the following model, based on the results obtained in this study:

$$AA_{out} \leftrightarrow AA_{in} \quad (1)$$

$$AA_{in} \leftrightarrow AA_{metab} \quad (2)$$

where Eq. 1 corresponds to distribution of AA between outer and intracellular space, i.e., between FABPs in medium and cellular FABPs *(14)*, and Eq. 2 reflects the involvement of AA into different metabolic pathways like incorporation in to phospholipids, oxidation, etc.

The first period of AA uptake in the presence of serum FABPs (0–2.5 h) (Fig. 1A) with the clearly defined plateau can be explained in terms of this model as a reaching of an equilibrium between outer and intracellular AA,

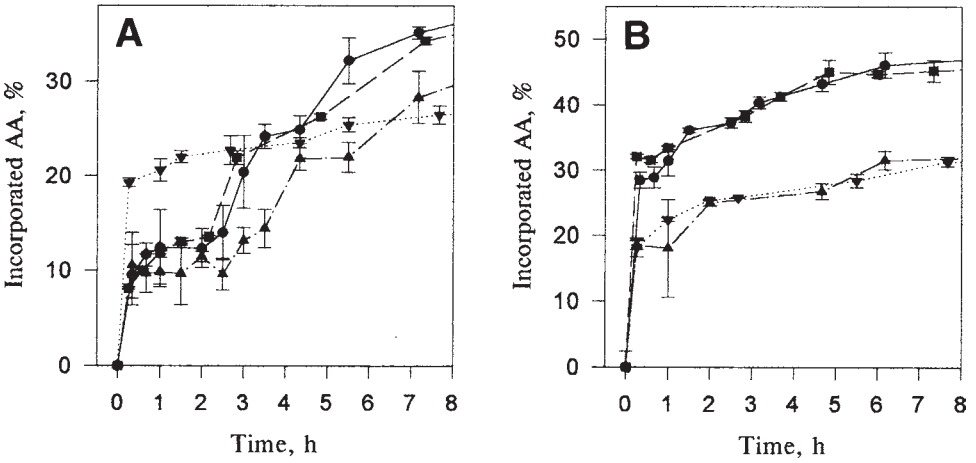

Fig. 2. The kinetics of [^{3}H]-AA incorporation to macrophages with serum FABPs **(A)** and without FABPs **(B)**. Initial AA concentrations, *M*: (●), 10^{-10}; (■), 10^{-7}; (▲), 10^{-6}; (▼) 10^{-5}.

and the second period corresponds to a lagged incorporation of AA into different phospholipid pools. In the absence of FABPs, AA uptake followed the kinetics without any kinks (Fig. 2B).

The serum albumin is an important albumin among serum FABPs. It is known that there are albumin-binding proteins on the cell surface *(15)*. Our results allow one to conclude that serum FABPs participate in AA uptake not only as transporters but they possibly influence AA membrane crossing and intracellular AA uptake. Two pathways of AA incorporation into macrophage lipids can be activated depending on AA concentration. These pathways differ by their capacity to incorporate AA *(16)*. Our results show that albumin can modify cell plasma membrane and affect AA uptake. Further elucidation of the details of AA intracellular incorporation is of crucial importance to understand the molecular mechanism of signal transduction and AA metabolism.

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