0273-2289/96/6101-02—0167$06.25

Ultralow Concentrations of Ibuprofen Activate Cell Prostaglandin Synthesis

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ABSTRACT

The interest in the prostaglandin (PG) synthesis by animal cells today grows steadily because of the difficulties in obtaining them by any other way. Murine peritoneal macrophages can under certain conditions synthesize large amounts of PGs. The effect of well-known nonsteroidal anti-inflammatory drug ibuprofen on PG synthesis by the cells using a high-performance liquid chromatography (HPLC) method with fluorescence detection of 4-bromomethyl-7-methoxycoumarin (BrMMC) derivatives was studied. In our case, the main metabolites were PGE_2 and PGF_{2a}. The PG synthesis activation effect was shown by ibuprofen concentrations in the 10^{-10}–$10^{-14}M$ range with the maximum effect at the $10^{-12}M$. In this case, the ibuprofen effect was comparable in value with the effect of the well-known cell PG synthesis activator—calcium ionophore A_{23187}.

Although the exact mechanism of such an effect is not clear at the moment, at low concentration, ibuprofen itself is able to activiate PG synthesis in murine peritoneal macrophages.

Index Entries: Prostaglandin; ibuprofen; biosynthesis; HPLC.

INTRODUCTION

Prostaglandins (PGs) are very important physiological substances that are widely used in medicine. Since their low concentrations in biological tissues and also multiple asymmetric carbon centers complicate

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both extraction and chemical synthesis, interest in the use of the enzymatic method of their synthesis in biotechnology has been aroused (*1*). The main problem for this approach is the inactivation of prostaglandin H synthase (PGHS)—the rate-limiting enzyme of conversion of arachidonic acid (AA) into PGs—during the course of the reaction (*2*). No such problem exists when PGs are synthesized by living cells, which are able to recover the enzyme. We have investigated exogenous and endogenous regulators of PGHS (*2,3*) and modulation of the AA conversion in the immune system cells (*4*). We have found that the well-known nonsteroidal anti-inflammatory drug ibuprofen caused enhanced activation of AA metabolism in murine peritoneal macrophages at the picomolar concentration range (*5*).

In this study, we have investigated the influence of ibuprofen on the release of PGs, which are a part of AA metabolites (AAM) in murine peritoneal macrophages. A high-performance liquid chromatography (HPLC) method was used, which, contrary to the commonly used radioimmunoassay method, allowed us to investigate all types of cell-synthesized PGs simultaneously.

MATERIALS AND METHODS

Cell Culture

Resident mouse macrophages were obtained by peritoneal lavage of untreated mice with sterile Dulbecco's Modified Eagle Medium (DMEM, Sigma), containing 2 m*M* L-glutamine (Sigma) and antibiotics. Cells were counted and plated onto 24-well plates (1.2–1.5 × 10^6 cells/well) in 1.5 mL DMEM with 10% heat-inactivated fetal calf serum (Flow). The viability of the cells, as judged by Trypan blue exclusion, was never below 96%. The cells were incubated for 2–3 h at 37°C in a humidified atmosphere of 95% air/5% CO_2, so that macrophages could be attached to the culture dishes. Then nonadherent cells were removed, and fresh DMEM (0.5 mL/well) containing 0.5% bovine serum albumin (BSA, Serva) was added. The cells were incubated for 1 h before AA metabolism stimulation. Adherent macrophages were cultured with stimuli for 2 h. Then the culture medium was removed and immediately used for the further detection.

Prostaglandin Determination

The HPLC method with fluorescent detection of 4-bromomethyl-7-methoxycoumarin (BrMMC, Sigma) derivatives has been developed for the determination of PGs.

Aliquots (0.5 mL) of cell supernatant were acidified with 1*N* hydrochloric acid to bring the final pH to 3.0 prior to extraction with ethyl acetate (4 mL). Specimens of the extract were left overnight with sodium sulfate added to remove possible water traces. Then the solvent was evaporated to dryness under a stream of nitrogen. The residues were dis-

solved in acetonitrile (100 μL), and the obtained test sample solutions were stored at −20°C.

The BrMMC solution (4 m*M*, 50 μL in acetonitrile), a dicyclohexyl-18-crown-6 solution (4 m*M*, 50 μL in acetonitrile), and potassium carbonate (1 mg) were added to the test sample solution. It was vortexed and kept at 50°C for 15 min in the dark. After cooling, 20 μl of the resulting mixture were injected into the chromatograph. Reverse-phase HPLC was performed with a C_{18} column. The sample was eluted with acetonitrile–water (3:2, v/v). The flow rate was maintained at 0.8 mL/min. The eluent was monitored by a spectrofluorimeter coupled to a chromato-integrator for measuring peak areas. The excitation wavelength was set at 313 nm; the emission wavelength was set at 370 nm.

BrMMC derivatives corresponding to the individual PGs have shown reproducible separate peaks, clearly visible in the chromatograms. The PG range comprised the time period from 7–15 min. The control BrMMC peak was also registered in this range between the PGF_{2a} and PGE_2 peaks.

RESULTS AND DISCUSSION

There have been many efforts to improve the determination of AAM in biological matrices. Chromatographic methods have been widely applied in the analysis of PGs. The HPLC method using fluorogenic BrMMC was suggested earlier (*6,7*), but it was not widely used, possibly because of it being unreproducible. The adding of a new component (dicyclohexyl-18-crown-6) into the reaction mixture and the subsequent employment of darkness for the derivatization procedure enabled us to obtain stable and reproducible results.

Murine peritoneal macrophages synthesize a wide range of PGs, namely PGE_2, PGI_2, PGE_1, PGF_{2a}, and TxB_2, which was demonstrated by a number of researchers (*8–10*). The quantitative and qualitative analysis data given in the above-mentioned works differ greatly, assuming it was owing to the cell-cultivating conditions. The synthesis of each PG is a two-enzyme process. The first step is the conversion of AA to intermediate PGH_2 by the action of PGHS. At the next stage, a number of other enzymes begin to participate. For example, endoperoxide-PGF_{2a}-reductase converts PGH_2 into PGF_{2a}, whereas endoperoxide-PGE-isomerase converts PGH_2 into PGE_2. However, there may exist a reductase that catalyzes the interconversion of PGE_2 and PGF_{2a}.

PGE_2 and PGF_{2a} were the main metabolites in our case. These PGs are produced by nonstimulated macrophages in noticeable quantities. Stimulating the cells by the widely used AAM metabolism activator—calcium ionophore A_{23187} (8 μ*M*)—leads to a twofold increase in PG quantity, whereas the PGE_2 and PGF_{2a} remain the main metabolites (Fig. 1A).

The adding of high concentrations of ibuprofen (10^{-4}*M*) to the cells fully inhibited the PG synthesis (Fig. 2) which is owing to PGHS activity

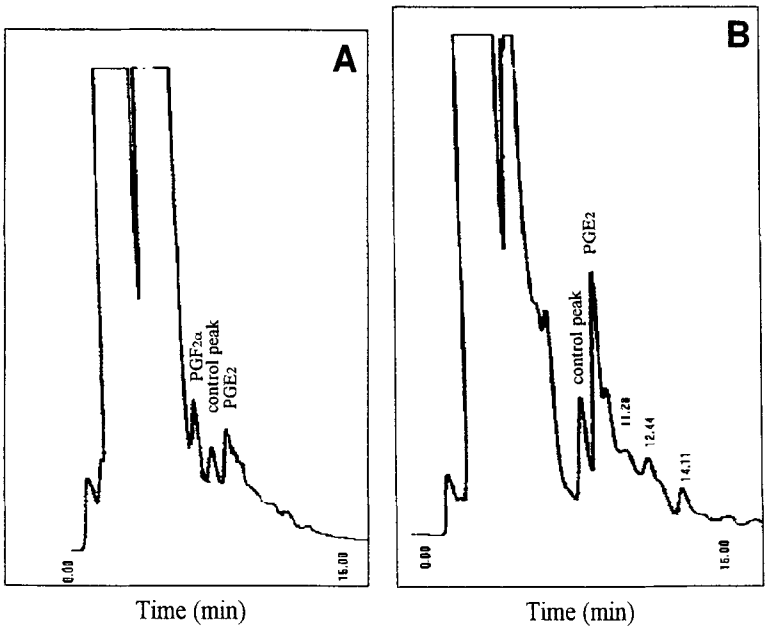

Fig. 1. Effect of addition of A_{23187}, 8 μM (**A**) and ibuprofen, $10^{-12}M$ (**B**) on the PG range HPLC elution profiles. Each chromatogram shown is the result of a representative experiment chosen from six (A) and three (B) similars. For experimental details, *see* Materials and Methods.

being inhibited (2). On the other hand, the use of low ibuprofen concentrations significantly increased the PG synthesis. The concentration increase effect of the PGs among all possible AAM was stable and reproducible. However, in three series of the experiments out of six, the highest increase was registered for PGE_2, whereas in the other three series it was PGF_{2a}. At the present stage, the mechanism of such a "switch" of the synthesis pathway remains unknown.

The PG synthesis activation effect was shown by ibuprofen concentrations in the 10^{-10}–$10^{-14}M$ range with the maximum effect at $10^{-12}M$ (Fig. 2). In this case the ibuprofen effect was comparable in value to the effect of A_{23187}. As we have shown earlier, ibuprofen activated the general metabolism of AA (5). The applied HPLC method made it possible to prove that the ibuprofen effect was directly linked to the PG synthesis. Detailed studies of the effect mechanism are currently under way.

ACKNOWLEDGMENTS

The study was financially supported by the 94-04-12703 grant from the Russian Fund for Fundamental Research.

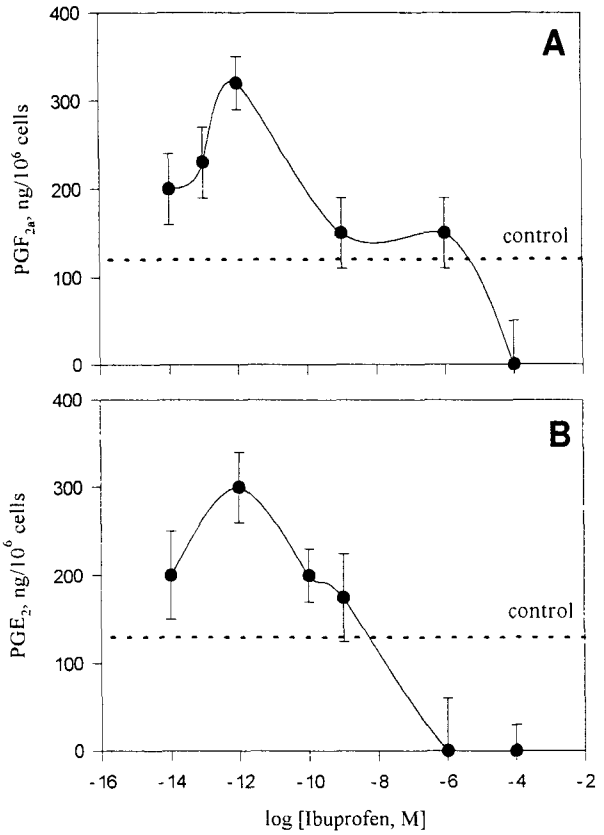

Fig. 2. Ibuprofen influence on the PGF_{2a} (**A**) and PGE_2 (**B**) release from the cells. The data shown are the results of a representative experiment, chosen from three similars. For experimental details, *see* Materials and Methods.

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