EFFECT OF PROSTAGLANDINS ON α-AMINOISOBUTYLIC ACID UPTAKE IN CULTURED FIBROBLASTS

Sei-itsu Murota and Mieko Kawamura

Department of Pharmacology, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo-173, Japan

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SUMMARY: Effect of various prostaglandins on the uptake of α-aminoisobutylic acid by cultured fibroblasts was studied. All the prostaglandins having an OH functional group in an intramolecular 5-membered ring showed an inhibitory effect on the amino acid uptake. The active compounds can be ranked in potency according to the values for the inhibition of the amino acid uptake per cent of control: prostaglandin $F_{2\alpha}$(53 %)>$F_{1\alpha}$(54 %)>D_2(56 %)>E_2(62 %)>thromboxane B_2 (66 %). Thus, prostaglandin $F_{2\alpha}$ was found to be the most potent inhibitor to membrane permeability and the inhibitory effect was dose dependent. The inhibition was maximal after 1 hour of exposure to prostaglandin $F_{2\alpha}$, persisted at least up to 6 hours in the presence of prostaglandin $F_{2\alpha}$.

In previous papers (1-3) we reported that prostaglandin(PG) $F_{2\alpha}$ had a specific stimulatory effect on the production of hexosamine-containing substances by cultured fibroblasts. During the course of the experiment of the mechanism of the effect, it was unexpectedly discovered that $PGF_{2\alpha}$ lowered the α-aminoisobutylic acid(AIB) uptake by the cells. AIB uptake most likely reflects the activity of the "A system" for the transport of naturally occuring amino acids, originally described for the Ehrlich cell by Oxender and Christensen (4). Further investigation disclosed that all the other prostaglandins having an OH functional group in an intramolecular 5-membered ring showed a similar inhibitory effect on membrane permeability. Hollenberg (5) has recently reported steroid-stimulated AIB uptake in cultured human fibroblasts. These findings suggest that biologically active lipids such as steroids and prostaglandins play some important role in regulating active transport by cell membrane and over all rates of macromolecule synthesis.

MATERIALS AND METHODS

[^{14}C]AIB (spec. act. 57 mCi/mmole) was purchased from the Radiochemical Centre, Amersham, England. PGA_1, A_2, B_1, B_2, D_2, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were kindly supplied from the Japan Upjohn Co. Ltd., Tokyo, Japan and PGE_2 was from Ono

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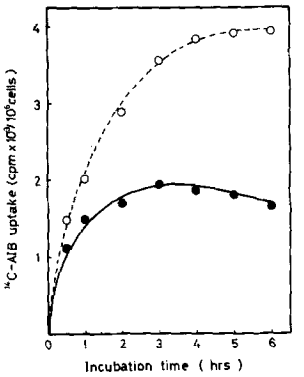

Figure 1. Effect of $PGF_{2\alpha}$ on the kinetics of AIB uptake by cultured fibroblasts. $PGF_{2\alpha}$ and [^{14}C]AIB were simultaneously added to the medium at the begining of the experiments. See the text for details.

Pharmaceutical Co. Ltd., Osaka, Japan. The thromboxane B_2 was the generous gift of Dr. John E. Pike, The Upjohn Co., Kalamazoo, MI, U.S.A. Arachidonic acid was purchased from Sigma Chemical Co., Missouri, U.S.A. Cloned fibroblasts, C 5, which were established by the authors (6, 7) were cultured as monolayer on a Petridish (35 mm diametor) with 1.5 ml of Ham's F 12 medium supplemented with 10 % of fetal bovine serum at 37°C in 5 % CO_2 atmosphere. The medium was changed every 2-3 days. The uptake of AIB was measured in intact confluent monolayers as described in a previous paper (7) with a slight modification. After 18-20 hours from the last renewal of the medium, the medium was transfered to 1 ml of fresh Ham's F 12 medium supplemented with 10 % dialized fetal bovine serum. To the medium each prostaglandin (or other compound to be tested) dissolved in ethanol was added. The final concentration of ethanol was always less than 0.1 % and usually well below 0.01 % (v/v), and always the same amount of vehicle was added to the control cultures. The uptake of [^{14}C]AIB (1.7 μM, 0.1 μCi/ml/culture) was measured during an appropriate period indicated in the text. All timed intervals, the medium was aspirated and the cell layer was washed quickly with two successive 2-ml rinses of ice-cold Ca^{2+} and Mg^{2+} free phosphate buffered saline pH 7.4 containing 5 mM unlabeled AIB as carrier. Cells were ruptured with 1 ml of distilled water and harvested to a counting vial with a rubber policeman. They were completely solubilized in a Triton X-100 containing scintilator; toluten (0.6 % PPO): Triton X-100 (2:1 by volume).

RESULTS AND DISCUSSION

The effect of $PGF_{2\alpha}$ on the kineticts of AIB uptake by cultured fibroblasts.

Cells in the stationary phase were simultaneously exposed to both 1 μg/ml of $PGF_{2\alpha}$ and [^{14}C]AIB for various length of time, and the activities of the cells in uptaking [^{14}C]AIB into the cells themselves during the corresponding period were compared with those of the control cultures. The results are shown in Fig. 1. In the control cultures, AIB was rapidly accumulated in the cell layer during the first hour, after which it kept increasing for 4 hours, though

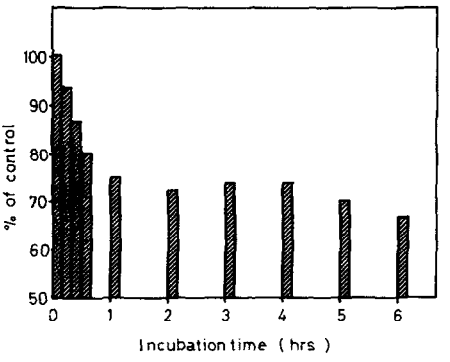

Figure 2. Time course of onset of $PGF_{2\alpha}$-inhibited AIB uptake. Cells were treated with $PGF_{2\alpha}$ for varying periods. [^{14}C]AIB was added to each cultures 10 min before each termination. See the text for details.

the accumulation rate gradually decreased. Finally, the increase leveled off after 4 hours. In $PGF_{2\alpha}$ treated cultures, however, saturation of AIB uptake appeared as early as 2 hours after the begining of the experiment and the level of AIB accumulation was only half of the control after that time. These data suggest that $PGF_{2\alpha}$ cause an alteration in the cell membrane, and that the alteration appeared as early as 30 min after exposure to $PGF_{2\alpha}$, and continued for at least 6 hours.

Rate of onset of prostaglandin action.

Cells in the stationary phase were exposed to 1 μg/ml of $PGF_{2\alpha}$ for varying periods and [^{14}C]AIB was added to the cultures 10 min before each termination. Cells were exposed to $PGF_{2\alpha}$ for the entire period of the experiment. Fig. 2 shows the time course of the onset of $PGF_{2\alpha}$-inhibited AIB uptake. Each value, shown as per cent of control, indicates the potency of the cells in uptaking AIB during 10 min periods. The inhibition of AIB uptake was found to begin as early as 10-20 min and it was not seen at all during the first 10 min at least. Maximal inhibition was observed after about 1 hour of exposure to $PGF_{2\alpha}$ and the inhibition persisted up to at least 6 hours. Comparing our data with the obser vations by Hollenberg(5,8,9),who reported that a 5 hour-lag time was required

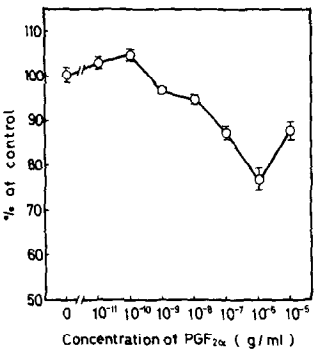

Figure 3. Dose response relationship for the effect of $PGF_{2\alpha}$ on AIB uptake by cultured fibroblasts. Cells were treated with $PGF_{2\alpha}$ for 1.5 hours plus 10 min with [^{14}C]AIB. See the text for details.

for the onset of glucocorticoid-stimulated AIB uptake and that the stimulation of AIB uptake by glucocorticoid as well as insuline and epidermal growth factor was blocked by cycloheximide, the alteration of membrane transport caused by $PGF_{2\alpha}$ took place much more rapidly. This suggests it is not due to protein synthesis but only due to physico-chemical change. $PGF_{2\alpha}$ does not seem to enter the cells or be metabolized by the cells, because when the cells were incubated with [^{3}H] $PGF_{2\alpha}$ for 24 hours, a negligible amount of radioactivity was measured in the cell layer and no radioactive metabolites were detect in either cell layer or medium.

Dose-response relationships for $PGF_{2\alpha}$.

Cells in the stationary phase were exposed to $PGF_{2\alpha}$ in a wide range of concentration for 1.5 hours, and [^{14}C] AIB was added to the cultures during the following 10 min. The concentration of vehicle ethanol totalled 0.1 % in each dosage, even in the control. Fig. 3 shows the dose-response curve of the cells to the prostaglandin. Inhibitory effect appeared first at the concentration of 1 ng/ml and the inhibition increased with increasing concentration up to 1 μg/ml. Some recovery was seen at the highest dose, which was a highly reproducible phenomenon and was difficult to explain at this stage. At the two lowest doses, i.e. 10 pg and 100 pg/ml which are about the reported limits of the

physiological concentration (10), some stimulatory effect was seen, though the stimulation was not statistically significant.

The effect of various prostaglandins on AIB uptake by cultured fibroblasts.

Cells in the stationary phase were exposed for 3 hours each to PGA_1, A_2, B_1, B_2, D_2, E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, arachidonic acid and TXB_2, one by one, at the concentration of 1 μg/ml each. At the same time [^{14}C]AIB was simultaneously added to the medium. The activities of the cells in uptaking [^{14}C]AIB during the 3 hours in treated cultures was compared with those of the control cultures. The results are shown in Table 1. All the compounds tested were found to have a tendency to inhibit AIB uptake by the cells, though no statistical significance was seen in the case of PGA_1, A_2, B_1 and arachidonic acid. $PGF_{2\alpha}$, $F_{1\alpha}$, D_2, E_2 and TXB_2, in descending order of potency, were the most potent compounds for eliciting this phenomenon. In the case of $PGF_{2\alpha}$, the rate of AIB uptake was only half of the control. The inhibitory effect of PGE_1, and B_2 was very mild. It is noteworthy that a big difference was seen between PGE_1 and E_2 in the effect on this index. PGE_2 showed more than 40 % inhibition, while PGE_1 showed only 7 %. Since the only difference in the chemical structure of these two prostaglandins was the unsaturated double bond located between C5-C6, this double bond must have something to do with the cause of the alteration of membrane transport. From the view point of molecular structure, the OH-functional group in the intramolecular 5-membered ring of prostaglandin must be focused on, because from the data in Table 1 it can be induced that having the OH-functional group in the 5-membered ring is an indispensable condition for inhibiting membrane permeability. Since prostaglandins are a kind of lipid, it is possible that they are able to interact with cell membranes. Hollwnberg (5) has recently reported a similar observation with steroids. Using the culture fibroblasts system, he found glucocorticoids having a stimulatory effect on AIB uptake, and from the data with steroid analogues, he has reported that the analogues lacking an 11β-substituent such as predonisone and cortisone neither stimulate the uptake of AIB nor block the action of active analogues.

Table I

The effect of various prostaglandins

on AIB uptake by cultured fibroblasts.

Treatment (1 μg/ml)	[^{14}C]AIB uptake (cpm/10^6 cells)	% of control	Significance
None	8039 ± 103.4 *	100.0	-
Arachidonic acid	7887 ± 85.3	98.1	n.s.
Prostaglandin A_1	7876 ± 209.6	98.0	n.s.
Prostaglandin A_2	7763 ± 170.0	96.6	n.s.
Prostaglandin B_1	7598 ± 343.0	94.5	n.s.
Prostaglandin B_2	7269 ± 213.4	90.4	$p<0.01$
Prostaglandin E_1	7514 ± 41.9	93.5	$p<0.05$
Prostaglandin E_2	4955 ± 77.5	61.6	$p<0.005$
Prostaglandin D_2	4513 ± 75.1	56.1	$p<0.005$
Prostaglandin $F_{1\alpha}$	4361 ± 115.3	54.2	$p<0.005$
Prostaglandin $F_{2\alpha}$	4266 ± 16.7	53.1	$p<0.005$
Thromboxane B_2	5291 ± 210.9	65.8	$p<0.005$

* mean ± S.E. (n=3)

According to Kram et al.(11), treatment which increases the cAMP concentration of cells, such as serum starvation, exogenous dibutyryl cAMP or PGE_1, causes inhibition of transport. From our findings, however, cAMP level in the cells does not necessarily have to do with membrane permeability, because $PGF_{2\alpha}$, which induces much less cAMP than PGE_1, showed a much greater inhibition of AIB uptake than PGE_1 (Table I). TXB_2 has recently proven to be formed from arachido nic acid via prostaglandin endoperoxides, i.e. PGG_2 and PGH_2, followed by another transformation via labile TXA_2 (12). TXA_2 has been reported to cause

strong platelet aggregation and vasocontraction (12-14). Recently we also found TXB_2 to be formed from arachidonic acid by inflammed tissue (15,16). The data in Table I shows TXB_2 is also a potent inhibiting substance for membrane transport, equal to $PGF_{2\alpha}$. Further investigation on the biological role of prostaglandins including TXB_2 is under way in our laboratory and will be reported elsewhere.

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