Vol. 83, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 28, 1978 Pages 679-687

EFFECT OF VARIOUS PROSTAGLANDINS ON THE RELEASE OF ARACHIDONIC ACID FROM CULTURED FIBROBLASTS

Sei-itsu Murota, Tokiya Yokoi, Ikuo Morita and Yo Mori*

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

Received April 6,1978

SUMMARY : Effect of various prostaglandins on the release of arachidonic acid from [^{14}C]arachidonic acid labeled fibroblasts was studied. Prostaglandin(PG) $F_{2\alpha}$ was found to enhance the release of radioactive arachidonic acid from the cells. The stimulatory effect was dose dependent, and was greater than that of bradykinin. The active compounds can be ranked in potency for the release of arachidonic acid from the pre-labeled cells per cent of control : $PGF_{2\alpha}$ (200.1%)>$PGF_{1\alpha}$ (141.8%)>PGD_2 (137.1%)>thromboxane B_2 (113.7%)>PGE_2 (109.4%). On the other hand, PGI_2 showed a strong inhibitory effect on the arachidonic acid release from the pre-labeled cells (the value was only 69% of the control), while 6-keto$PGF_{1\alpha}$, an end metabolite of PGI_2, had no effect.

In a previous paper (1), we reported that $PGF_{2\alpha}$ and other prostaglandins having an OH-group in an intramolecular 5-membered ring had an inhibitory effect on α-aminoisobutylic acid uptake in cultured fibroblasts. During the course of an experiment on the mechanism of the effect, it was discovered that $PGF_{2\alpha}$ enhanced the release of arachidonic acid (and PGE_2) from [^{14}C]arachidonic acid labeled fibroblasts in culture. These findings combined with our other series of experiments showing that $PGF_{2\alpha}$ has a specific stimulatory effect on the production of hexosamine-containing substances by cultured fibroblasts (2-5), suggest that $PGF_{2\alpha}$ alters membrane constitution and regulates cell activity.

MATERIALS AND METHODS

[^{14}C]arachidonic acid (specific activity 60.2 mCi/mmol) was obtained from the Radiochemical Centre, Amersham, England. Arachidonic acid and bradykinin triacetate were purchased from Sigma Chemical Co.,Missouri,U.S.A. Prostaglandin A_1, A_2, B_1, B_2, D_2, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were kindly supplied by the Japan Upjohn Co., Ltd., Tokyo, Japan. Prostaglandin E_1, $F_{1\beta}$, $F_{2\beta}$, I_2 methyl ester

* Department of Biochemistry, Tokyo College of Pharmacy, Hachioji-shi, Tokyo, Japan

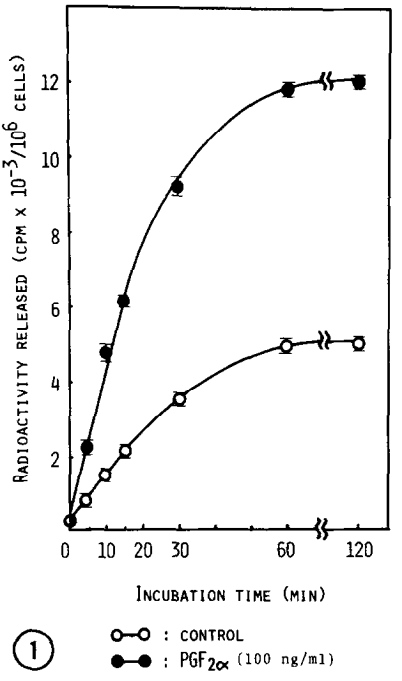
0006-291X/78/0832-0679$01.00/0

and 6-ketoprostaglandin $F_{1\alpha}$ were from Ono Pharmaceutical Co., Ltd., Osaka, Japan. Thromboxane B_2 was the generous gift of Dr. John E.Pike, the Upjohn Co., Kalamazoo, Mich., U.S.A. Cloned fibroblasts, C-5, which were established by the authors from 15-day-old carrageenin granuloma of rat (6,7) were cultured as monolayer on a Petri-dish (35 mm diameter) with 1.5 ml of Ham's F12 medium supplemented with 10% of fetal bovine serum at 37°C in 5% CO_2 atmosphere. Under these conditions, generation time of the cells was about 20.4 hrs. The [^{14}C]arachidonic acid labeled cells were prepared by cultivating the fibroblasts at the stationary phase with medium containing [^{14}C]arachidonic acid (0.1 μCi/ml/dish) for 18-24 hrs. After labeling, the medium was removed and the cell layer was quickly washed twice with 1 ml of serum free medium warmed at 37°C. The [^{14}C]arachidonic acid labeled fibroblasts were used for the following release experiment. The labeled cells were exposed for 1 hr to 1 ml of Ham's F12 medium supplemented with 10% dialyzed fetal bovine serum plus one of several stimuli. Each stimulus was added to the medium as a ethanol solution. The final ethanol concentration of the culture medium 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. After the 1 hr cultivation, each medium was collected in a tube and centrifuged at 900 x g for 15 min to remove any contamination of free cells or cell debris. 0.5 ml of the supernatant was carefully transfered to a counting vial and the radioactivity was measured by a liquid scintillation counter. The composition of the scintillation cocktail was 0.6% PPO containing toluene/triton X-100 (2:1, v/v).

RESULTS AND DISCUSSION

When the cells were labeled with radioactive arachidonic acid for 18 hrs, about 96% of the radioactivity was incorporated into the cells. For analysis of the incorporated arachidonic acid, the lipids of the labeled cells were extracted and chromatographed by the method of Folch et al.(8). About 71% of the radioactivity was associated with phosphatidylcholine, 25% was associate with phosphatidylethanolamine, and the rest was due to trigrycerides and free arachidonic acid.

[^{14}C]arachidonic acid labeled cells were exposed to fresh medium containing 100 ng/ml of $PGF_{2\alpha}$ for various lengths of time, and the radioactivity released from the cells to the medium during corresponding period was compared with that of the control cultures. The results are shown in Fig. 1. In the control cultures, radioactivity was linearly released from the cells during the first 15 min, and the releasing rate gradually decreased during the following 45 min and then leveled off. In $PGF_{2\alpha}$ treated cultures, the amount of released radioactivity was always more than 2 times that of the control cultures throughout the experimental period, though the releasing pattern was the same as in the control. The alteration appeared from the beginning and



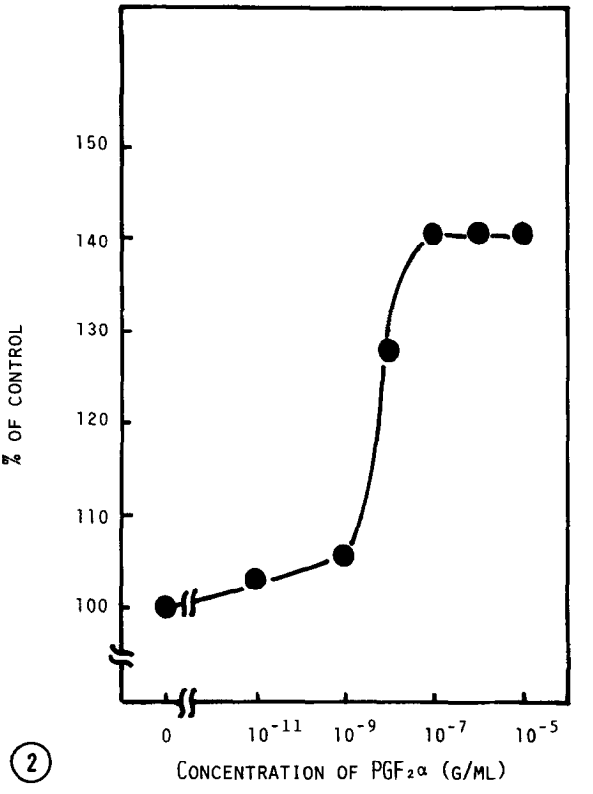


Fig. 1 Effect of $PGF_{2\alpha}$ on the kinetics of radioactivity release from [^{14}C]arachidonic acid labeled fibroblasts. (n=3).

Fig. 2 Dose response relationship for the effect of $PGF_{2\alpha}$ on radioactivity release from [^{14}C]arachidonic acid labeled fibroblasts.

became significant as early as 5 min after exposure to $PGF_{2\alpha}$ and continued for at least 2 hrs. The radioactivity released was extracted with ethyl acetate and subjected to thin layer chromatography with authentic arachidonic acid, PGD_2, PGE_2, $PGF_{2\alpha}$, 6-keto$PGF_{1\alpha}$ and thromboxane B_2. The radiochromatogram of the thin layer plate, developed with a solvent system (ethyl acetate/iso-octane/acetic acid/water, 110:50:20:100, v/v), showed that 90% of the radioactivity was due to [^{14}C] arachidonic acid and the rest was due to [^{14}C] PGE_2 in both the $PGF_{2\alpha}$ and the control cultures. Providing further confirmation that the largest amount of radioactivity released was radioactive arachidonic acid, the major radioactivity co-migrated with authentic arachidonic

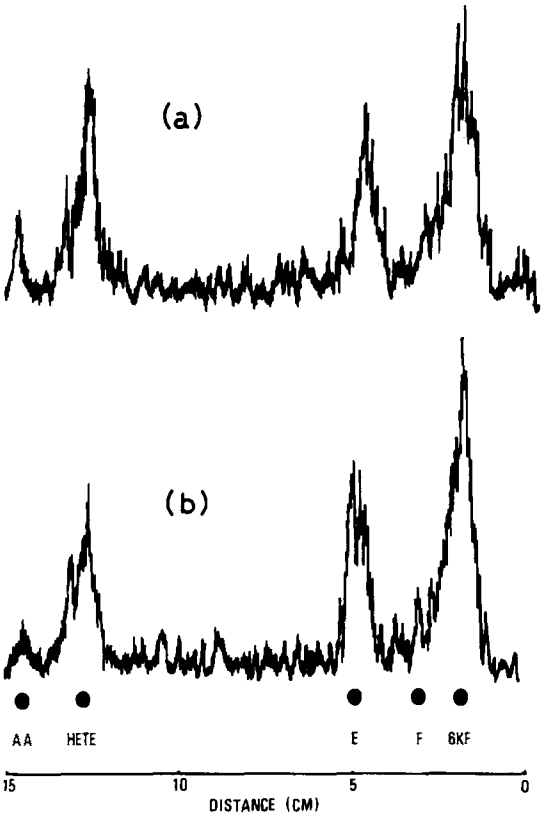


Chart 1. Radiochromatogram scan showing the prostaglandin synthesizing activity by the microsomes of bovine seminal vesicle with (a) released radioactivity from pre-labeled cells, (b) authentic [^{14}C]arachidonic acid. AA; arachidonic acid, E and F; prostaglandin E_2 and $F_{2\alpha}$, 6KF; 6-ketoprostaglandin $F_{1\alpha}$. See the text for details.

acid was extracted and incubated with bovine seminal vesicle microsomes plus boiled 105000 x g supernatant of bovine seminal vesicle. Under these conditions, more than 95% of the radioactivity was converted to compounds co-migrating with authentic hydroxy arachidonic acid : HETE (Rf=0.78), PGE_2 (Rf=0.30), $PGF_{2\alpha}$ (Rf=0.15) and 6-keto$PGF_{1\alpha}$ (Rf=0.10) in the solvent system of ethyl acetate /iso-octane/acetic acid/water (110:50:20:100, v/v) with the conversion rates of 20,25,3,50%, respectively (chart 1). These results are in good agreement

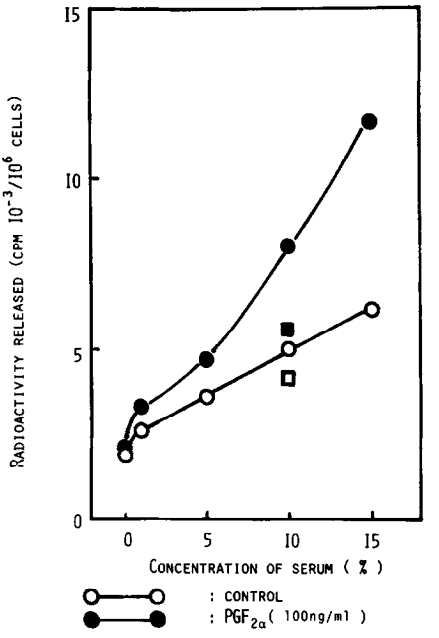


Fig. 3 Effect of serum concentration on the release of radioactivity from [^{14}C]arachidonic acid labeled fibroblasts. circles : using dialyzed serum, squares : using normal serum.

with those in our previous paper (9) describing prostaglandin synthesis in bovine seminal vesicle and the paper reported by Cottee et al.(10) using ram seminal vesicle. The fact that most of the radioactivity released was due to radioactive arachidonic acid is consistent with the data reported by Hong and Levine (11) and Newcombe et al.(12) who used pre-labeled mouse fibroblasts and human synovial fibroblasts, respectively.

The pre-labeled fibroblasts were exposed to $PGF_{2\alpha}$ in a wide range of concentrations for 1 hr and the radioactivity released from the cells to the medium was measured. The concentration of vehicle ethanol totaled 0.01% in each dosage, even in the control. Fig. 2 shows the dose-response curve of the cells to the prostaglandin. A stimulatory effect appeared first at the concentration of 10 ng/ml and stimulation reached maximum level arround the concentration of 100 ng/ml.

Since serum has been reported to be a potent releaser of arachidonic acid

from cell membrane (11), the relationship between $PGF_{2\alpha}$ and serum in the stimulation of arachidonic acid release was examined. [^{14}C]arachidonic acid labeled cells were exposed to medium containing 100 ng/ml of $PGF_{2\alpha}$ plus different amounts of dialyzed fetal bovine serum for 1 hr and the radioactivity released from the cells to the medium was compared with that of the series of the control cultures. The results are shown in Fig. 3. The stimulatory effect of $PGF_{2\alpha}$ on arachidonic acid (plus PGE_2) release increased with the increasing concentration of serum (at least up to 15%). This might be explained if we assume that serum protein acts as a "trap" for the released arachidonic acid, since the amount of arachidonic acid released is transient and exceeds that require for prostaglandin synthesis, and the excess would presumably be immediately reincorporated into phospholipids and could not be detected, unless there is enough serum to act as a "trap". With respect to this, Isakson et al.(13) have recently succeeded in detecting arachidonic acid release following hormonal stimulation by utilizing bovine serum albumin as a "trap" for released arachidonic acid from isolated perfused organs.

The effect of $PGF_{2\alpha}$ was compared with that of bradykinin, since bradykinin has been found to stimulate the release of arachidonic acid and PGE_2 by selectively activating a phospholipase A_2 without affecting cyclo-oxygenase and subsequent enzymes (14). As shown in Fig. 4, the addition of bradykinin in various doses to the culture medium led to a dose-dependent increase in the release of radioactivity from the pre-labeled fibroblasts. The maximum increase by bradykinin was obtained at the concentration of 5×10^{-6}M, however, the increase was only 70% of that caused by $PGF_{2\alpha}$. Recently, Blackwell et al. (15) showed that 1 μg of bradykinin stimulated phospholipid hydrolysis by 150-300% using guinea pig isolated lung. Our data suggest that $PGF_{2\alpha}$ can also affect phospholipase.

[^{14}C]arachidonic acid labeled cells were separately exposed for 1 hr each to PGA_1, A_2, B_1, B_2, D_2, E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, $F_{1\beta}$, $F_{2\beta}$, I_2 methyl ester , 6-keto-$PGF_{1\alpha}$, arachidonic acid and thromboxane B_2 at the concentration of 100 ng/ml

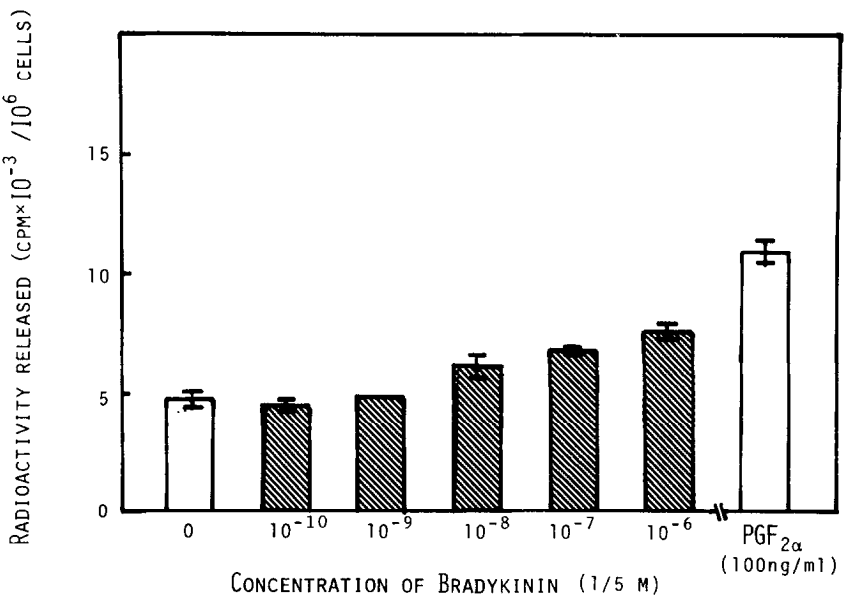


Fig. 4 Comparison of the effect of bradykinin with that of $PGF_{2\alpha}$ on radioactivity release from [^{14}C]arachidonic acid labeled fibroblasts. (n=3)

each. The effect of each stimulus on the arachidonic acid (plus PGE_2) release from pre-labeled fibroblasts was compared with that of the control cultures. The results are shown in Table I (n=3). $PGF_{2\alpha}$ was found to be the most potent prostaglandin on this index. $PGF_{1\alpha}$ and PGD_2 showed half as much potency as $PGF_{2\alpha}$. It is noteworthy that a large difference was seen between the α and β series of PGF in the effect on this index. The configration of the OH-group in an intramolecular 5-membered ring must be an important factor in this effect. The stimulatory effect of PGE_2 and thromboxane B_2 was mild. The release of arachidonic acid or prostaglandin has been discussed in respect to the accumulation of cyclic AMP, since most of the prostaglandin releasers such as bradykinin, histamine and epinephrine concomitantly elevate the level of cyclic AMP in the tissue (12,16). Our findings, shown in Table I, clearly demonstrate that the cyclic AMP level in the cells has nothing to do with the release of arachidonic acid in a direct way, because $PGF_{2\alpha}$ showed a much more greater stimulation of arachidonic acid release than PGE_2, which induces

TABLE I

THE EFFECT OF VARIOUS PROSTAGLANDINS ON RELEASE OF RADIOACTIVITY FROM ^{14}C-ARACHIDONIC ACID LABELED FIBROBLASTS

Treatment (100ng/ml)	Radioactivity released (cpm/10^6 cells)	% of control	Treatment (100ng/ml)	Radioactivity released (cpm/10^6 cells)	% of control
None	4225 ± 100.4	100.0			
AA	4359 ± 28.6	103.1	PGF1α	5992 ± 17.7 **	141.8
PGA1	4269 ± 3.5	101.0	PGF2α	8458 ± 91.8 **	200.1
PGA2	4325 ± 22.4	102.3	PGF1β	4114 ± 159.7	97.4
PGB1	4259 ± 122.3	100.8	PGF2β	4283 ± 240.0	101.4
PGB2	4189 ± 183.2	99.1	PGI2	2944 ± 165.9 **	69.7
PGD2	5794 ± 87.9 **	137.1	6kF1α	4417 ± 204.7	104.5
PGE1	4112 ± 104.9	97.3	TXB2	4805 ± 196.2 *	113.7
PGE2	4623 ± 30.6 *	109.4	† Cortisol	4102 ± 270.5	97.3

* Statistical significance : $P < 0.05$

** Statistical significance : $P < 0.005$

AA : Arachidonic acid

TXB_2 : Thromboxane B_2

6KF1α : 6-ketoProstaglandin F1α

† Cortisol (1 μg/ml)

much more cyclic AMP than $PGF_{2\alpha}$ but had no effect on the release of arachidonic acid at all. Regarding this, Newcombe et al.(12) have demonstrated that bradykinin dose not directly activate adenylate cyclase activity in a particulate fraction derived from human synovial fibroblasts.

On the other hand, PGI_2 methyl ester showed a strong inhibitory effect on the arachidonic acid release from the cells, while 6-ketoPGF$_{1\alpha}$, an end metabolite of PGI_2, had no effect. Other prostaglandins,such as PGA_1, A_2, B_1 and B_2 had no effect at all. From these facts, we might speculate that the regulation of prostaglandin production is effected by prostaglandin themselves, presumably by the activation or inhibition of phospholipase either directly or indirectly.

Hong and Levine (11) demonstrated that cortisol inhibits arachidonic acid release from pre-labeled mouse fibroblasts in culture. Under our conditions, however, cortisol failed to show the inhibitory effect on arachidonic acid

release. There have been conflicting reports regarding on the effect of corticosteroids on the release of prostaglandins from various organs (17). With regards to this problem, Grodzinska and Dembinska-Kiec (18) have recently demonstrated that pre-treatment with cortisol is necessary to the inhibitory effect of cortisol.

A study of the ubiquity of the effect of $PGF_{2\alpha}$ on arachidonic acid release showed that $PGF_{2\alpha}$ failed to show the effect at least 3 other cell lines tested, i.e., 3T3, 3T6 and Hela, suggesting that the effect of $PGF_{2\alpha}$ might be specific to fibroblasts originating from inflamed tissue.

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