SELF REGULATION OF GROWTH BY HUMAN DIPLOID FIBROBLASTS VIA PROSTAGLANDIN PRODUCTION

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1. Introduction

The prostaglandins comprise a family of related compounds which are capable of modulating a number of cellular functions including cell division [1–3]. These fatty acid-derived molecules are either extremely unstable in aqueous solution or are quickly metabolized, in vivo, to biologically less active or inactive substances [1]. The hormone-like action of these substances, therefore, it short range, generally on cells located in the vicinity of the site of their production.

In this communication we report that prostaglandin (PG) production by human diploid fibroblasts in culture serves to enhance or inhibit their growth. We show that indomethacin and aspirin, when added to these cultures, initially inhibit and later stimulate cell growth while inhibiting prostaglandin production. We further note that PGE_2 added to these cells, at concentrations endogenous to these cultures, inhibits cell division while $PGF_{2\alpha}$ stimulates cell growth. Surprisingly, the effect on cell growth of hydrocortisone resembles that of indomethacin and aspirin. We find hydrocortisone also inhibits prostaglandin production in human diploid fibroblasts.

2. Materials and methods

Human embryo lung fibroblasts (IMR90) were grown and maintained in culture in basal medium, Eagle (BME) containing 10% fetal calf serum (FCS) and 50 units/ml penicillin, 50 μg/ml streptomycin. The cells were passaged and brought to quiescence as described previously [3]. To obtain a new round of division in the quiescent cultures, the cultures were fed with fresh medium containing 10% FCS. The prostaglandins were placed into aqueous solution with sodium bicarbonate. The inhibitors of prostaglandin synthesis were solubilized with alcohol. Prostaglandin E was determined as the sum of E, A and B by radioimmunoassay [4]. The conversion to prostaglandin B was accomplished by treatment of the medium with NaOH. $PGF_{2\alpha}$ was determined by radioimmunoassay directly. Interference of the assay by medium was determined and corrected for. The accuracy of the PG values was confirmed by the addition of known PG quantities to the test samples.

3. Results

Prostaglandins, added to quiescent human diploid fibroblasts in culture, simultaneously with fresh medium including fresh serum, will either enhance or inhibit the subsequent round of cell division, depending on the type of prostaglandin added. This is illustrated in table 1. PGE_2 at a final concentration of 5×10^{-8} M or 5×10^{-9} M inhibits cell division. $PGF_{2\alpha}'$ on the other hand, enhances cell growth at 5×10^{-8} M.

The addition of inhibitors of PG production to human diploid fibroblasts, influences the growth of these cultures. We were able to demonstrate this effect repeatedly on low passage IMR90 fibroblasts. Figures 1 and 2 are based on results from a typical experiment. Figure 1 demonstrates that cell growth in the presence of inhibitors of PG synthesis, is slowed during the log

Table 1
Effect of added PGE_2 and $PGF_{2\alpha}$ on cell growth

Addition to culture	Increase of cells/cm²	% Inhibition	% Stimulation
None	18 077 ± 570	–	–
5×10^{-8} M PGE_2	13 872 ± 348	23%	–
5×10^{-9} M PGE_2	14 755 ± 280	18%	–
5×10^{-10} M PGE_2	18 285 ± 213	–	–
5×10^{-8} M $PGF_{2\alpha}$	21 395 ± 573	–	18%

Cells were plated on 35 × 10 mm culture dishes and allowed to become quiescent as previously described [4]. At the start of the experiment, three dishes were washed with 1 ml Ca^{2+}, Mg^{2+}-free phosphate buffered saline, treated with 1 ml 0.25% trypsin solution and the resulting cell suspension was counted with a Coulter Counter. Fresh medium with or without prostaglandin was added to triplicate dishes. Forty-eight hours later the cultures were treated as described above and cell number was again determined with a Coulter Counter. Results are expressed as ± SEM.

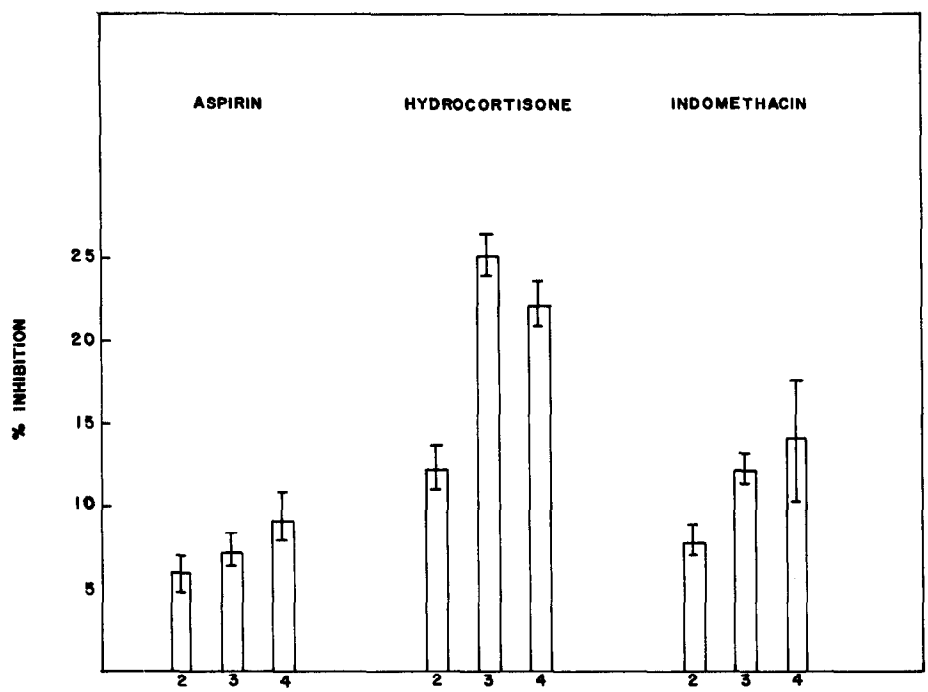

Fig.1. Inhibition of growth in the log growing cells. HDF (IMR90) were plated at a density of 5 × 10^3 cells/cm² on 35 × 10 mm culture dishes. Four hours after plating the medium was removed and fresh medium with or without aspirin, indomethacin or hydrocortisone (5×10^{-6} M) was added. At this point five plates were washed once with 1 ml Ca^{2+}, Mg^{2+}-free phosphate buffered saline, treated with 1 ml 0.25% trypsin solution and counted using a Coulter Counter. Forty-eight hours after drug addition (day 2) duplicate plates from each group were counted. Following day 2, the cells were counted each day and fed fresh medium with or without drugs every 48 h.

phase of growth (day 2–4). Figure 2 illustrates that after day 4, as cell proliferation decreases due to increasing cell density, the presence of two of the inhibitors (aspirin and hydrocortisone) in the cultures stimulates growth.

To assess the influence of indomethacin, aspirin and hydrocortisone on prostaglandin synthesis in our culture system, we added them to the human diploid fibroblast cultures for 48 h and then determined the presence of prostaglandin in the medium. The results, as shown in table 2, indicated that 5×10^{-6} M aspirin, hydrocortisone or indomethacin inhibit prostaglandin production by human diploid fibroblasts, 80%, 85% and 100% respectively. Indomethacin and aspirin inhibit fatty acid cyclo-oxygenase, the enzyme catalyzing the first step of prostaglandin biosynthesis, and therefore inhibit the production of subsequent metabolities [5]. Hydrocortisone has been shown by Hong and Levine to block the conversion of the phospholipid precursor to the free fatty acid substrate in methylcholanthrene transformed 3T3 cells [6]. Control levels of PGE are approximately 1.5×10^{-9} M (table 2). This value is in the range of concentrations of PGE_2 which effect cell division when added to freshly stimulated quiescent cultures (table 1).

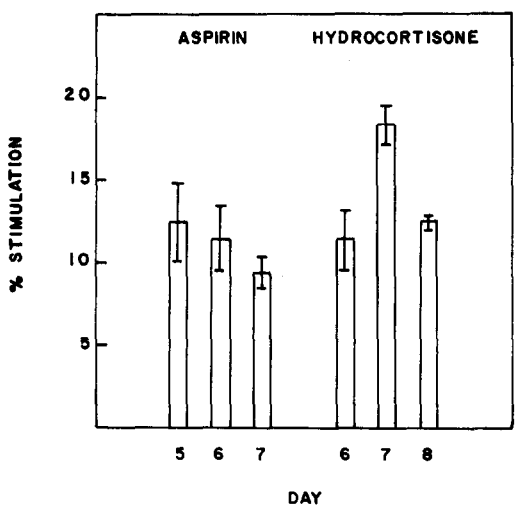

Fig.2. Stimulation of growth in human diploid fibroblasts. A continuation of experiment depicted in fig.1. See fig.1 for experimental details.

Table 2
Inhibition of PG production in HDF

Addition to culture	Sum of PGs A, E and B (pg/ml)
None	567
5×10^{-6} M Aspirin	115
5×10^{-6} M Hydrocortisone	84
5×10^{-6} M Indomethacin	0

Cells were plated on 35 × 10 mm culture dishes at a density of 5×10^3 cells/1.5 ml medium/dish and fed every 48 h. On the sixth day, cells were fed medium containing 5×10^{-6} M aspirin, hydrocortisone or indomethacin. Forty-eight hours after drug addition, medium was removed from 2 plates/group and pooled (3 ml total). The sum of prostaglandins E, A and B was determined by radioimmunoassay. PG levels in medium incubated for 48 h in the absence of cells was determined and subtracted to obtain above values.

4. Discussion

In sum, our results demonstrate that endogenously produced prostaglandins function as regulators of cell division in human diploid fibroblasts. These cells in culture have a finite life span and at the time tested were approx. 13–17 population doublings from the original explant.

The inhibition of prostaglandin production in these cultures has a biphasic effect on cell growth. It remains to be determined what combination of factors and which prostaglandins function in this control mechanism. To date prostaglandins of the E-type have been associated with the inhibition of cell proliferation [2,3], while $PGF_{2\alpha}$ has been linked to the stimulation of growth [7]. Our own results demonstrate this point at endogenous prostaglandin concentrations (table 1), in quiescent fibroblasts restimulated to divide with fresh serum. PGE, however, can also stimulate cell division. Bem and Greaves demonstrated this with PGE_1 in mouse epidermal cells [8]. Feher and Gidali showed that PGE_2 will stimulate stem cell proliferation [9]. The prostaglandin type produced, the quantity of prostaglandin produced and the responsiveness of the cells to the prostaglandins, particularly at different cell densities, may all prove to be interacting variables.

These results were obtained at high serum concen-

trations (10%), those normally used to maintain these cells in culture. It is likely that at lower serum concentrations, the influence on cell division by these endogenously produced prostaglandins will be accentuated [7].

Our observations may have important implications as to the growth condition of human diploid fibroblasts in vivo. Fibroblasts are normally quiescent in the organism. Following trauma, the fibroblasts proliferate, produce collagen and then once again become quiescent. The prostaglandins are known to be produced following trauma [10]. These substances may thus be involved in normal wound healing.

The studies of Hayashi and Sato [11], Jimenez DeAsua and Rozengurt [12], and others indicate that positive and negative control of cell growth in many cell types is probably a result of multi-effector action. Our results suggest that the prostaglandins make up a segment of this effector system. A cellular autoregulatory role for the PGs may also exist in other cell types. For example, Thomas et al. showed that the rate of PGE production is inversely related to the growth rate of the continuous cell lines, HeP-2, L and HeLa [13].

The effect of hydrocortisone on cell growth and prostaglandin production is also of interest. Hydrocortisone has been shown by others to promote cell growth in many mesodermally derived cell lines [14–16] and to inhibit growth in other cell lines [17]. We have shown that hydrocortisone can promote or inhibit growth of human diploid fibroblasts depending on when it is added to the culture and that this effect may, at least in part, be due to influence on prostaglandin synthesis.

Acknowledgement

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