Competitive Growth Stimulation by Ca\(^{++}\) and the Platelet-Derived Growth Factor in Human Diploid Fibroblasts

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ABSTRACT. The platelet-derived growth factor (PDGF), a potent mitogen for normal human fibroblasts, shows reduced activity in medium containing regular levels of Ca\(^{++}\). A double reciprocal plot of the growth rate of IMR-90 cells versus the Ca\(^{++}\) concentration in the presence or absence of PDGF showed a common intercept on the ordinate, which gives a minimum doubling time of 20 h for middle aged cells. This is evidence of a competitive growth stimulation by PDGF and Ca\(^{++}\) that differs from the non-competitive mode of action of EGF by which is also reduced the requirement of extracellular Ca\(^{++}\) for normal human cell growth.

The platelet-derived growth factor (PDGF) is a potent serum growth factor with a unique mode of action on cell proliferation. PDGF stimulates G0-arrested Balb/c 3T3 cells to become 'competent' to enter the S phase (8) and prevents replicating cells from entering G0 (11). However, stimulation of growth by PDGF is not evident in normal human fibroblasts when the culture medium contains an adequate amount of Ca\(^{++}\) (10). To detect clear growth stimulation by PDGF, the Ca\(^{++}\) concentration should be reduced to less than 0.3 mM (10). Therefore, PDGF and Ca\(^{++}\) may stimulate growth by acting competitively on cells, which we here report is the case.

Normal human fetal lung fibroblasts, IMR-90, were maintained as described (7) with TOM-H medium, a modified MEM with 39 additives such as non-essential amino acids, vitamins, heavy metals, lipids, nucleic acids, and HEPES (7), containing 10% fetal bovine serum (FBS). Ca\(^{++}\) deficient TOM-H medium was prepared by omitting CaCl\(_2\) from the TOM-H medium. Nevertheless, this medium contained 0.004 mM of Ca\(^{++}\) derived from Ca-pantothenate and Ca-folinate. To prepare Ca\(^{++}\) deficient serum, we dialyzed 50 ml of FBS against 0.01 M sodium phosphate buffer, pH 7.4, containing 0.08 M NaCl by passing it through a Zeineh dialyzer (Zeineh Co. Ltd.). NaCl powder (73 mg) was added to half the amount of the dialyzed serum. This serum then was used as dialyzed FBS after passing it through a conventional membrane filter. The other half of the dialyzed serum was applied to a CM-Sephadex C50 column (100 ml gel bed volume) equilibrated with sodium phosphate buffer. The column then was washed with 220 ml of this buffer. The break-through fraction combined with the wash was concentrated to the original volume on a PM-10 membrane (Amincon

Abbreviations: EGF, epidermal growth factor; FBS, fetal bovine serum; PDGF, platelet-derived growth factor; PDL, population doubling level; ΔPDL, increase in PDL; TOM-H medium, an extensively modified minimal essential medium (7).
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This concentrate, to which 73 mg of NaCl was added, was used as the non-cationic dialyzed FBS. PDGF was partially purified by the method of Antoniades et al. (1) up to the step of cation-exchange chromatography. The final protein concentration of the preparation used in the experiment was 19.2 μg/ml, as determined by A280 using BSA as the standard protein. Poly-L-arginine was purchased from Seikagaku Kogyo Co. and polyethyleneimine from Tokyo Kasei Kogyo Co. Chick erythrocyte inner histone was the gift of Drs. M. Zama and K. Mita of National Institute of Radiological Sciences. The cell growth rate was measured by seeding 2,200 cells suspended in the TOM-H medium with 10% FBS in a well of a Falcon 3008 dish (Falcon Plastics Co.). After overnight incubation in a humidified CO2-incubator, the cells were washed twice with 0.5 ml of Ca++, Mg++-free phosphate buffered saline. Samples (0.5 ml) of the Ca++ deficient TOM-H containing 10% non-cationic dialyzed FBS and an appropriate amount of Ca++ and/or PDGF were added to each well, then the cells were cultured for 4 days. The cell number per well was counted with a hemocytometer after trypsinization. The increase in the population doubling level (ΔPDL) for 4 days was calculated from the cell number per well after washing with Ca++, Mg++-free phosphate buffered saline.

To determine whether Ca++ and PDGF act competitively, we measured the growth rate of human diploid fibroblasts, IMR-90, at the 37 population doubling level (PDL) at various concentrations of Ca++ in the presence or absence of PDGF. Growth stimulation by PDGF was apparent for a wide range of concentrations of Ca++, although in the higher ranges stimulation was less (Fig 1a). The double reciprocal plot of the two curves, i.e., 1/[Ca++] vs. 1/[growth rate], showed good linearity and a common intercept on the ordinate (Fig. 1b). According to the kinetic theory of growth response

![Fig. 1](image)

**Fig. 1.** Growth of IMR-90 cells at various concentrations of Ca++ and its competitive modulation by PDGF. IMR-90 cells at 37 PDL were cultured in the control medium containing 10% non-cationic dialyzed FBS (see text). The growth rate is shown as ΔPDL over 4 days. Each point is the mean of duplicate cultures. The maximum deviation of each measurement was less than 5.5% of the mean.

- (●) Control, (○) + PDGF.
- (a) Growth rate of IMR-90 cells.
- (b) Double reciprocal plot of the growth rate versus the Ca++ concentration. Data are derived from (a). The intercept on the ordinate (arrow), 0.209, gives a minimum doubling time of 20 h for middle aged IMR-90 cells.
Competitive Action of Ca++ and PDGF

The cellular response to serum fits a model which is analogous to the Michaelis-Menten formulation. Therefore, our finding indicates that action of PDGF is competitive with that of Ca++. The maximum growth rate calculated from the intercept is 4.8 PDL/4 days; i.e., 20 h per population doubling for middle aged IMR-90 cells in their limited replicative life span (7). This maximum growth rate, however, was not observed in control cultures, presumably because of the inhibitory action of Ca++ above 1 mM (Fig. 1a). The negative intercept on the abscissa in Fig. 1b gave the Km. The Ca++ concentration required for half-maximal growth was reduced to 1/4.7 with a concentration of PDGF.

Since both PDGF and Ca++ are cationic substances, the above action may be due to their cationicity as in the case of the basic polymers described by Murakami and Yamane (6). However, the other cationic substances shown in Table 1 could not be substituted for 1 mM Ca++ or PDGF. Sr++, the cation closest to Ca++ in the biological system, stimulated cell growth to a considerable extent, but not to the level of Ca++. Poly-L-arginine and polyethyleneimine, even at the optimal concentration of 1 µg/ml, did not stimulate growth in spite of their potent action on tumor cells (6). These results suggest that growth stimulation is attributable to Ca++ and PDGF, but not to the non-specific cationicity of the ion or the growth factor.

Our present observations show apparent differences in the relationship between the epidermal growth factor (EGF) and Ca++ in that EGF reduces the extracellular Ca++ requirement for normal human cell growth (4, 5) by acting unidirectionally; it reduces the Ca++ requirement by more than 50-fold, whereas Ca++ does not affect the Km of EGF. As Pledger et al. (8) reported, PDGF stimulates the growth of Balb/c 3T3 cells by activating G0-arrested cells to become 'competent' for DNA synthesis. Ca++ also can induce this 'competence', but EGF acts on the 'progression' of G1; the step following 'competence' (12). This difference probably contributes to the different modes that regulate the extracellular Ca++ requirement of normal human cells.

### TABLE 1. EFFECTS OF CATIONIC SUBSTANCES ON THE GROWTH RATE OF IMR-90 CELLS.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Concentration</th>
<th>ΔPDL/4 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>1.27</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1 mM</td>
<td>3.82</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1 mM</td>
<td>2.13</td>
</tr>
<tr>
<td>SrCl₂</td>
<td>1 mM</td>
<td>2.89</td>
</tr>
<tr>
<td>PDGF prep.</td>
<td>19.2 µg/ml</td>
<td>3.93</td>
</tr>
<tr>
<td>Histone*</td>
<td>1.0 µg/ml</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>19.2 µg/ml</td>
<td>1.88</td>
</tr>
<tr>
<td>Poly-L-arginine</td>
<td>1.0 µg/ml</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>19.2 µg/ml</td>
<td>1.65</td>
</tr>
<tr>
<td>Polyethyleneimine</td>
<td>1.0 µg/ml</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td>19.2 µg/ml</td>
<td>-0.27**</td>
</tr>
</tbody>
</table>

IMR-90 cells at 38 PDL were cultured in TOM-H medium containing 10% non-cationic dialyzed FBS and 0.1 mM CaCl₂ as the basal Ca++ level to give comparable growth rates for the cells combined with 1 mM CaCl₂ and those combined with PDGF. Values are the means of triplicate cultures.

* Chick erythrocyte inner histone.

** Apparent toxic effect.
The competitive action seen between Ca\(^{++}\) and PDGF suggests that these two growth stimulators may have a common site of action. It may be located on the cell surface because PDGF is a high molecular peptide and Ca\(^{++}\) can bind not only to the cytosolic binding protein, calmodulin (3), but to the cell surface as well (9).

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**REFERENCES**


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