Control of the proliferation and differentiation of GEJ under platelet aggregating factor treatment

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The growth and the differentiation of thyroid cells is known to be under the control of both cyclic AMP dependent and independent pathways. Taking into account these data, we studied the regulation of proliferation and the expression of differentiation using the human thyroid hybrid cells we cloned (Karsenty et al. 1985). This study was conducted using platelet aggregating factor-acether (PAF-acether) (Roubin et al. 1983), a phospholipid mediator which has been shown to stimulate the phosphoinositol cycle of porcine thyroid cells without promoting cAMP accumulation (Haye et al. 1984).

Materials and Methods

Reagents
Highly purified human TSH (hTSH) was supplied by the National Pituitary Agency (Baltimore, MO) and iodine labelled using the lactoperoxidase method.
PAF-acether was a gift of Dr J. M. Mencia-Huerta.
Bovine serum albumin (BSA) and insulin were purchased from Sigma (St. Louis, MO).

GEJ cell culture and proliferation
GEJ cells were maintained in complete culture medium: RPMI 1640 (Gibco, Paisley, Scotland), penicillin 100 U/ml, streptomycin 100 μg/ml and 10% heat inactivated foetal calf serum (FCS) in an atmosphere of 10% CO2 in air. For proliferation, cells were seeded at 5 × 10^3 cells (in 0.2 ml complete medium with 5% FCS) in a 96 well microtest II Falcon tissue culture plate and PAF-acether added at the specified concentrations. Cells were counted at the required time with a hemocytometer.

Binding assay of [125I]TSH to GEJ cells was performed as previously reported (Roubin et al. 1983).

Results
Five thousand GEJ cells were cultured with 10^-7 M to 10^-12 M PAF-acether in complete culture medium containing 5% FCS. On days 3, 5 and 7 viable cells were counted with a haemocytometer. As shown in Table 1, on day + 3 of culture, an increase of approximately 60% in cell numbers was found with doses of PAF-acether varying from 10^-9 to 10^-11 M. This increase promiferative response of the GEJ cells induced by the PAF-acether was still detected on day + 5 while it disappears on day + 7 of culture.

In a second series of experiments, we investigated if PAF-acether, apart from its proliferative action, could influence the number of binding sites for TSH on GEJ cells. From preliminary experiments (data not shown), we determined that incubation of GEJ cells during 1 h with 10^-12 M PAF-acether increases of approximately 50 to 60% the numbers of binding sites for TSH, this effect being detectable but less intense after 30 min of incubation. Moreover, we demonstrated that similar amounts of BSA have no effect on the number of TSH-R on GEJ cells (Table 2). We also investigated if these PAF-acether induced binding sites were specific for TSH; for that purpose, GEJ
Table 1.
Effect of PAF-acether dilutions on GEJ cell numbers (mean of 3–4 experiments).

<table>
<thead>
<tr>
<th>Day of culture</th>
<th>PAF-acether dilutions (M)</th>
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<tbody>
<tr>
<td></td>
<td>10^{-7}</td>
</tr>
<tr>
<td>3</td>
<td>6.6*</td>
</tr>
<tr>
<td>5</td>
<td>6.7</td>
</tr>
<tr>
<td>7</td>
<td>4.8</td>
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* × 10^3 per well of culture.

Table 2.
Specificity for TSH of PAF acether induced TSH receptors on GEJ cells (mean ± SEM of 3 determinations).

<table>
<thead>
<tr>
<th>Competitive protein (× 2500)</th>
<th>One hour incubation with 10^{-12} M of</th>
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<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>992 ± 28</td>
</tr>
<tr>
<td>TSH</td>
<td>414 ± 26</td>
</tr>
<tr>
<td>Insulin</td>
<td>1044 ± 44</td>
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cells were incubated with high amounts of unlabelled TSH or insulin simultaneously to incubation with [^{125}I]TSH and we found that only unlabelled TSH was able to abolish [^{125}I]TSH binding to GEJ cells while insulin was not able to do so (Table 2).

In a third set of experiments, we studied the affinity of these TSH binding sites detected on GEJ cells after 1 h incubation with 10^{-12} M of PAF-acether. For that purpose, two saturation curves were established with GEJ cells previously treated or not with 10^{-12} M PAF-acether, these cells being further incubated with 10 ng of [^{125}I]TSH and varying concentrations of unlabelled TSH (1 to 10 µg). As shown in Fig. 1, binding of [^{125}I]TSH on PAF-acether pre-treated GEJ cells occurs at low concentrations of unlabelled TSH indicating that most of the PAF-acether induced binding sites exhibit a high affinity for hTSH.

Fig. 1.
Specific binding of [^{125}I]TSH to GEJ after pre-incubation without • or with o 10^{-12} M PAF-acether.
Discussion

Permanent cultures of differentiated cells offer many advantages in studying regulatory mechanisms of proliferation and differentiation. Contrasting with the FRTL-5 cell line (Ambesi-Impiombato et al. 1980), GEJ cells which are hybridoma cells are independent of TSH for proliferation. These last years, numerous factors such as dibutyryl-cyclic AMP, insulin, epidermal growth factors (EGF) or phorbol myristate acetate have been reported to influence various cell growth; most of these factors trigger the hydrolysis of phosphatidylinositol. Compared to them, PAF-acether act through diacylglycerol formation and activation of protein kinase C (Haye et al. 1984) independently of the cAMP pathway. Concerning thyroid cell growth, all these factors and TSH were shown to induce thyroid cell proliferation.

From these experiments, it is clear that PAF-acether significantly augments GEJ cell numbers. However, it must be noted that this effect was obtained in complete medium containing 5% FCS, while it never occurs in complete culture medium free of FCS, indicating that undefined serum factors are necessary to GEJ cell proliferation. A similar effect of PAF-acether on proliferation of hemopoietic cell line was recently reported (Roger & Dumont 1984); the authors demonstrated that 24 h after the addition of 10^-9 to 10^-7 PAF-acether, a significant reduction of the doubling time was noted, this effect being obtained in presence or absence of FCS but fibrinogen being necessary to PAF-acether effect. The effect of PAF-acether on the GEJ cell differentiation evaluated in terms of binding sites for [125I]TSH occurs very quickly, 1 h after addition of PAF-acether and at a very low concentration (10^-12 M). These conditions are quite comparable to the specific effect of PAF-acether on platelets. The effect of this agent is similar to that of EGF, a growth factor also acting through diacylglycerol pathway (Haye et al. 1984), which was shown to induce thyroid cell proliferation of quiescent cells and to reverse the expression of differentiation in TSH treated cells (Roger & Dumont 1984).

Lastly, another interesting effect of PAF-acether is the induction of high affinity. TSH binding sites on GEJ cells, once more a similar effect of PAF-acether was reported for binding of [125I]fibrinogen on Raji cells (Levesque et al. 1986). However, the number of TSH-binding sites on GEJ cells remains low (around 2500 binding site per GEJ cell) even after PAF-acether treatment.

References


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