Presence and Possible Mode of Action of a Proteinaceous Gonadotropin-like Growth Regulating Factor in Plant Systems

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Abstract. Antiserum to human chorionic gonadotropin (HCG) caused marked inhibition of adventitious rooting of Begonia semperflorens and Chrysanthemum morifolium stem cuttings. Immuno-absorption of crude protein extract from chrysanthemum foliage through a column of polymerized and insolubilized HCG antibodies resulted in a significant reduction in adventitious root promoting activity of the extract. These results are discussed in the light of a hypothesis that an endogenous protein growth regulating substance which immunologically resembles HCG exists in plant systems. Further experimentation with HCG suggests that its mode of action is possibly via the regulation of peroxidase enzymic control of auxin levels.

In previous studies Leshem (10) and Leshem and Lunenfeld (11) have shown that HCG, a proteinaceous human sex hormone, has growth stimulating effects on certain morphogenetic processes in plants, especially upon adventitious rooting of Vitis vinifera, Begonia semperflorens and Brassica oleracea. It was shown that HCG possibly acts via regulation of endogenous gibberellin levels, the latter being markedly repressed by the gonadotropin, and a hypothesis was suggested that HCG triggers mevalonic acid conversion to steroids thereby diminishing precursor for gibberellins.

In the present study by use of immunological techniques an attempt has been made to indicate the existence of an endogenous gonadotropin-like proteinaceous growth regulating factor in plants. Further experimentation was carried out in order to elucidate the mode of HCG action in plant systems, especially in the light of hormone-enzyme interactions which are prevalent both in plant (3, 7, 13, 14, 15, 21) and in animal (8, 17) systems.

Methods

Rooting studies on effects of HCG, HCG antiserum and serum upon stem cuttings of Begonia semperflorens cv. Indian Maid and Chrysanthemum morifolium cv. Indianapolis were performed using the technique and experimental conditions described previously (11), only this time with the root culture nutrient solution described by Street and Henshaw (20). The HCG antiserum, prepared in rabbits, was supplied by the Institute of Endocrinology of the Tel-Hashomer Government Hospital, Israel.

One ml of the antiserum neutralized 600 International Units (IU) of HCG as determined by the procedure of Loraine and Brown (12). As control active rabbit serum lacking HCG antibodies was used.

The approach used in the immuno-absorption trials was as follows: If a protein extract of plant material were to be passed through a medium containing HCG antibodies, the effluent would be depleted of any existent endogenous HCG or any immunologically similar antigenic substances which could "cross react" with the antibodies (1) and resultingly evoke a weaker growth stimulating response. This system was obtained by insolubilizing HCG antibodies by covalent binding of the molecules to one another with ethyl-chloroformate using the technique described by Avramaes and Ternynck (2). The immunoabsorbent properties of such insolubilized proteins were found by these workers to be specific, stable and effective to isolate specific antigens from mixtures and to remove contaminating antigen proteins from solutions. The protein obtained in this manner was packed in a 50 cm × 3 cm glass column through which protein extract was subsequently passed. In order to determine whether this procedure does actually work in the present trials, various concentrations of HCG solutions were run through test columns and resulting effluents were assayed for presence of HCG by immuno-electrophoresis, ring tests and spectrophotometric readings. In all cases no HCG was detected.

Crude foliage protein extract of Chrysanthemum morifolium cv. Indianapolis was obtained from 100 g fresh weight of plants grown during the summer in a virus-free nursery at Mishmar Hashiva, Israel. Temperature and photoperiodic conditions were identical to those in previous trials (11). Foliage was homogenized in the cold with 0.1 M tris buffer pH 9, passed through a gauze filter to remove coarse matter and the homogenate centrifuged for 20 min.

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in a cooled Sorvall centrifuge. Super saturation of the supernatant was accomplished with \((\text{NH}_4)_2\text{SO}_4\) and subsequently recentrifuged for 15 min to obtain the "salted out" protein. The precipitate was dissolved in tris buffer pH 7.8 and dialyzed against the same buffer medium in order to remove all traces of \((\text{NH}_4)_2\text{SO}_4\). Dialysis was performed in the cold for 48 hr and solutions changed every 8 hr. The protein solution obtained was lyophilized and 0.5 g of aliquots taken for column separation.

In the experiment concerning possible auxin (IAA) and HCG interactions, growth responses were assayed by the Wheat Straight Growth test (9) using the modifications suggested by Sirois (19). The buffer was applied alone, with the addition of \(10^{-4}\) horse radish peroxidase (HRP) or with 25 mg/l IAA. In treatments the incubation medium contained \(10^{-5}\) mM MnCl\(_2\) and \(10^{-3}\) M H\(_2\)O\(_2\) which are promotive to peroxidase action (5,6).

Results

Effect of HCG Antiserum on Rooting. Results presented in table I in which mg dry weight root production in Begonia as affected by anti HCG, normal serum and HCG in table II where average root number and total length in Chrysanthemum in response to HCG antiserum and serum show that, in all parameters measured, the HCG antiserum produced significantly lower figures than the serum control.

Effect of HCG Immunoabsorption of Chrysanthemum Foliage Protein Extracts Upon Promotion of Rooting. Aliquots of 0.5 g protein extract were dissolved in buffer tris pH 7.8, passed through the anti HCG insolubilized protein immunoabsorption column and effluent collected. Results presented in table III compare adventitious rooting response of Chrysanthemum stem cuttings whose bases were immersed for 48 hr at 25° in either effluent solution or in "complete" protein extract in buffer before immunoabsorption. pH of effluent was essentially identical to that of the initial solution. After immersion cuttings were arranged in a randomized block design and grown under the same experimental conditions as described above.

From table III it is seen that in all parameters measured the effluent has statistically lower activity than the unabsorbed extract. This experiment was repeated and produced essentially similar results.

Auxin Related HCG Effects. The possibility that HCG acts on peroxidase and may thereby effect growth indirectly via auxin regulation was tested by incubating wheat coleoptile sections in a given concentration of exogenous IAA and HRP with and without HCG.

Results summarized in table IV show that HCG significantly reverses the HRP-inhibited elongation.

Discussion

The results presented in tables I and II show that HCG antiserum has a marked inhibitory effect on adventitious rooting of both Begonia and Chrysanthemum. The inhibiting effect cannot be attributed to other serum components since control with normal serum produced significantly higher results. This indicates the possibility that the HCG antibodies in

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Table I. Effect of HCG Antiserum, Serum, and HCG Upon Dry Weight Root Production of Begonia semperflorens Growing in Nutrient Solutions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mg/dry wt root production</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCG antiserum 1.25 ml/l</td>
<td>1.0</td>
</tr>
<tr>
<td>Normal serum 1.25 ml/l</td>
<td>8.3</td>
</tr>
<tr>
<td>Nutrient solution alone</td>
<td>9.1</td>
</tr>
<tr>
<td>HG 750 IU/l</td>
<td>56.0</td>
</tr>
<tr>
<td>Level of significance(^2)</td>
<td>(&lt;0.05)</td>
</tr>
</tbody>
</table>

\(^1\) Neutralizes 750 IU/l HCG.

\(^2\) According to test of Monotonic Association (16).

Table II. Effect of HCG Antiserum and Serum on Root No. and Length of Chrysanthemum morifolium Cuttings Growing in Nutrient Solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg no. of roots per cutting</th>
<th>Total cm root length per cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCG antiserum 1.25 ml/l</td>
<td>0.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Normal serum 1.25 ml/l</td>
<td>2.0</td>
<td>27.0</td>
</tr>
<tr>
<td>HG 750 IU/l</td>
<td>3.9</td>
<td>47.0</td>
</tr>
<tr>
<td>Level of significance(^1)</td>
<td>(&lt;0.05)</td>
<td>(&lt;0.05)</td>
</tr>
</tbody>
</table>

\(^1\) According to Mann-Whitney Test as described by Siegel (18).

Table III. Effect of Immuno-absorption Through Anti-HCG Antibody Insolubilized Protein on Adventitious Root Promoting Activity of Chrysanthemum Foliage Protein Extracts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avg no. of roots per cutting</th>
<th>Total cm root length per cutting</th>
<th>Total mg dry weight root production/cutting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein extract</td>
<td>9.0</td>
<td>36.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Anti-HCG column effluent</td>
<td>5.0</td>
<td>16.0</td>
<td>0.8</td>
</tr>
<tr>
<td>L.S.D. (&lt;0.05)</td>
<td>3.0</td>
<td>17.6</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Table IV. Effect of HCG on HRP Mediated Elongation of Wheat Coleoptile Growth in 25 mg/l Solutions of IAA

Relative growth as determined by the Wheat Straight Growth Test. Four replicate means of 10 coleoptiles each were used for each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Relative elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA 25 mg/l</td>
<td>152</td>
</tr>
<tr>
<td>IAA 25 mg/l + HRP 10^-6 M</td>
<td>100</td>
</tr>
<tr>
<td>IAA 25 mg/l + HRP 10^-6 M + HCG 500 IU/l</td>
<td>142</td>
</tr>
<tr>
<td>L. S. D. p&lt;0.05</td>
<td>11.4</td>
</tr>
</tbody>
</table>

the antiserum neutralize some factor immunologically similar to the antigenic HCG which is promotive in adventitious rooting (10, 11).

This hypothesis may be supported by the results in table III where it is seen that immunoabsorption of crude protein on HCG antibody insolubilized protein causes a marked reduction of all parameters of adventitious rooting measured in *Chrysanthemum morifolium*. A probability however exists that the column absorbed factors other than those immunologically similar to HCG and thereby produced lower growth promoting results. However, the specificity shown in similar systems by Avramaes and Ternynck (2) favors the former postulate.

The possibility of a hormone-enzyme interaction in growth regulation by HCG is indicated by results given in table IV. These results show that in addition to the possible GA-steroid metabolism linked mode of action indicated previously (11), HCG may effect the IAA-IAA peroxidase system which has been outlined by Galston et al. (6) and Galston (5). It is seen that the growth repression of wheat coleoptiles when incubated with HRP and IAA is significantly reversed by HCG. The action of the gonadotropin in this respect resembles the effect of diphenols on the above IAA-enzyme interaction (4).

Acknowledgments

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Literature Cited