Inhibition of Gibberellic Acid Biosynthesis by Chorionic Gonadotrophin During Cereal Grain Germination

It has been suggested[1,2] that a gonadotrophin-like growth regulating factor may be present in plants. It was proposed that this factor might be involved in the regulation of endogenous gibberellin levels since these were reportedly lowered in the presence of chuman horionic gonadotrophin (HCG). However, they were assayed by treating embryoless barley endosperm halves with extracts and measuring reducing sugar release[3]. Intrinsically this method is not entirely satisfactory[4,5] and, since it was found at the same time that HCG would inhibit reducing sugar release in response to gibberellic acid (GA), the exact significance of the results must be open to doubt. On the other hand, since no polypeptide or glycoprotein hormone-like substance has been reported in plants, it seemed worthwhile to clarify the situation. For this purpose the germinating barley grain was chosen since in it gibberellin synthesis and secretion (in the embryo) are readily separated from gibberellin action (on the aleurone).

The barley used was a sample of Proctor, dehusked by treatment with 50% H_2SO_4 and stored at 4 °C. Whole grains or 2 mm endosperm slices were weighed in groups of 10 and incubated for 26 h at 25 °C in 4 ml of solution as indicated below. 1 ml *M* NaCl was added to the solutions before homogenizing in a glass (hand) homogenizer. The homogenates were left to stand for 1 h at room temperature before centrifuging (MSE bench centrifuge, 5 min speed 10). α-Amylase activity in the supernatant was assayed at 25 °C by the iodine-dextrin colour method of BRIGGS[6].

The results, tabulated below, indicate the relative activities of α-amylase released in terms of the rate constants for the reaction and expressed in arbitrary units (AU) per g fresh weight as described by DUFFUS[7].

Human chorionic gonadotrophin, Stock No. CG-B from the Sigma Chemical Corporation at a specific activity of 2800 IU/mg was used. This preparation contained no additives but its low specific activity indicates that it may contain albumin and denatured HCG as impurities.

The results show that HCG at a concentration of 7 IU/dish will inhibit α-amylase production in intact germinating grains by 50%. Inhibition is virtually complete at a concentration of 140 IU/dish. Gibberellic acid dependent α-amylase synthesis in 2 mm endosperm slices is not inhibited by levels of HCG up to 280 IU/dish. α-amylase activity is unaffected by HCG.

Since HCG has no effect on the stimulated rate of α-amylase synthesis in intact grains incubated with GA_3, where the GA_3 must be transported from the micropile to the aleurone, it may be concluded that transport of GA_3 from the embryo to the aleurone layer is not inhibited. HCG must therefore inhibit either the synthesis or secretion of GA_3.

This is not the first report of an animal hormone eliciting a biochemical response in plants since ecdysone, the insect growth hormone stimulates growth in dwarf pea seedlings in a manner similar to that of GA_3[8]. There may well be others and it is possible that bioassays much cheaper and simpler than those currently in use for animal hormones might be devised using plant tissues. A correlation between the various activities of the hormone would have to be demonstrated in this event.

So far no specific factor controlling GA biosynthesis in plants has been described. Environmental factors such as light and cold influence GA biosynthesis but not with any degree of specificity. BRIAN[9] has postulated that GA biosynthesis is controlled in vivo by the phytochrome system. It is unlikely however, that phytochrome is uniquely concerned in GA biosynthesis as it has been implicated in a wide range of physiological phenomena.

If, as we suggest, a regulatory substance similar to HCG and controlling GA biosynthesis, is present in germinating barley then it may form part of a general mechanism governing GA levels in plants. For example, dwarfing of plants, frequently a result of a lowered GA production, may be caused by an overproduction of this HCG-like regulatory substance. Other basic plant processes in which GA is known to be concerned, such as abscission[10], senescence[11] and dormancy[12] may also involve this substance.

Relative activities of α-amylase released in terms of the rate constants for the reaction in intact barley grains and endosperm slices

Material	Addition	α-amylase activity in AU/g fresh wt.[a]
Intact grains	Distilled water	0.128 ± 0.02
	+ 140 IU[b] HCG	< 0.02
	+ 70 IU[b] HCG	0.05 ± 0.02
	+ 42 IU[b] HCG	0.04 ± 0.02
	+ 14 IU[b] HCG	0.064 ± 0.03
	+ 7 IU[b] HCG	0.061 ± 0.035
	+ 3 IU[b] HCG	0.108 ± 0.03
	5×10^{-8} *M* GA_3	0.294 ± 0.08
	+ 280 IU HCG	0.308 ± 0.08
Endosperm slices	Distilled water	< 0.02
	5×10^{-8} *M* GA_3	0.126 ± 0.01
	+ 280 IU HCG	0.115 ± 0.02
	+ 140 IU HCG	0.129 ± 0.03
	5×10^{-8} *M* GA_3 + 280 IU HCG added just before assay	0.126 ± 0.02

Each result is the mean of at least 3 experiments ± S.D. [a] AU, arbitrary units. [b] IU, international units of HCG.

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Finally, it would be interesting to know the effect of HCG on GA production by *Fusarium moniliforme*, the original source of GA[13]; if inhibition occurs, conceivably this may prove the ideal system for elucidating the mode of action of HCG at the molecular level[14].

Résumé. La gonadotrophine chorionique peut arrêter la production de l'α-amylase pendant la germination des grains intacts d'orge. Par contre, la synthèse de l'α-amylase controllée par l'acide gibberellique dans les sections de grains d'orge sans embryon n'est pas modifiée. La gonadotrophine chorionique doit donc arrêter la synthèse ou la sécrétion de l'acide gibberellique.

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Hepatic and Renal Neutral Angiotensinases

Although neutral angiotensinase activity has been found in various tissues, it is not clear how many enzymes are involved. The properties of plasma angiotensinases are well documented[1]; they appear to be different from those of erythrocyte angiotensinases[2] but similar to those of kidney angiotensinases[3]. Little is known about liver angiotensinases. The location of kidney angiotensinase in microsomes[3,4] and of liver angiotensinase in the cell sap[5] suggests the possibility of two different enzymes. The purpose of this paper is to further define and compare the properties of the neutral angiotensinases in liver, kidney and erythrocytes.

Materials and methods. Subcellular fractions of rat tissues were prepared according to the method of Ragab et al.[6] for the lirer and to that of Shibko and Tappel[7] for the kidney. Erythrocytes were separated from rat blood taken with disodium ethylenediamine tetracetic acid (EDTA-Na_2) as anti-coagulant. After washing twice with 0.9% saline, erythrocytes were hemolyzed by adding an equal volume of distilled water and centrifuged. The supernatant was diluted to an appropriate concentration with distilled water. Samples of liver cell sap, kidney microsomes or hemolysate, which contained 0.8 mg, 0.1 mg and 2 mg of protein, respectively, were incubated with 0.25–0.5 µg of angiotensin II or its analogues in 4 ml of 0.125 *M tris*-acetic acid buffer (pH 7.5) at 37 °C for 30 min. One part of the samples was dialyzed against EDTA-Na_2 solution (0.22% in 0.9% NaCl) and then against 0.9% NaCl (2.5 m*M*). Dithiothreitol (DTT) (1 m*M*), *p*-chloromercuriphenyl-sulfonic acid (PCMS), di-isopropylfluorophosphate (DFP) (1 drop of a 5% solution in w/v isopropanol), or (5 m*M*) $CaCl_2$ was added to the incubation mixture as activator or inhibitor. After removal of proteins by boiling and centrifugation, the remaining angiotensin was bioassayed in the rat, using [Asn[1], Val[5]]-angiotensin II as standard. Angiotensinase activity was defined as percentage of angiotensin inactivated during incubation.

Results and discussion. The Table shows effects of inhibitors and activators on angiotensinase activity of liver, kidney and erythrocytes on [Asn[1], Val[5]]-angiotensin II. Values are percentages of the activity compared to that of untreated samples.

Angiotensinase activity of these 3 kinds of samples was inhibited by dialysis against an EDTA solution and restored by addition of $CaCl_2$ but was not inhibited by DFP. The activity of liver cell sap and that of hemolysate were also inhibited by PCMS whereas microsomal angiotensinase of the kidney was resistant to this agent. Dithiothreitol prevented this inhibition.

Inhibition and activation of angiotensinase activity at pH 7.5

	PCMS	PCMS + DTT	EDTA	EDTA + Ca	DFP
Liver cell sap	0	94	0	78	111
Kidney microsome	97	102	9	98	99
Hemolysate	0	87	0	90	100

Itskovitz and Miller[2] showed that mercury compounds inhibited most of the angiotensinase activity of hemolysate but not of plasma. Our observation on the erythrocyte enzyme is consistent with their findings. Kokubu et al.[8] reported that a purified erythrocyte angiotensinase which cleaved the 2–3, 4–5 and 5–6 bonds of angiotensin was inhibited by either EDTA or DFP. They also stated that the inhibitory action of EDTA was not reversed by calcium or other divalent cations. This discrepancy from our observations could be explained by the existence of more than one enzyme in the hemolysate[9] or by differences in purity. Liver cell sap and hemolysate inactivated both [Asp[1], Ile[5]]-angiotensin II and [Asn[1], Val[5]]-angiotensin II, but had little effect on [Arg[1], Ile[5]]-angiotensin II.

Thus, the angiotensinase activity of the liver cell sap is similar to that of hemolysate but different from that

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