

vals and differences of means were calculated using Student's t-test at $p \leq 0.05$.

Results. Both the treatments caused sex inversion (table). Whereas AgNO_3 was able to cause male flower formation in all the female plants 30 days after treatment, GA_3 was able to do so only in half the number of plants. The difference in the number of nodes showing male flowers between the treatments was statistically insignificant on all days except day 5. The effect of treatment with AgNO_3 was more persistent. It delayed the resumption of production of female flowers by 10 days over GA_3 treatment. Normal male flowers are pedicellate and are borne on pedunculate cymose inflorescences. The tepals of the male flowers are typically reflexed at anthesis. The male flowers induced by AgNO_3 are sessile and form close clusters at each node as the inflorescence axis does not elongate. The flowers are otherwise similar to normal male flowers and set viable pollen. GA_3 causes elongation of the flower stalks and the flowers are smaller than normal male flowers, but set viable pollen. During the induction of male flowers, as well as at the time of formation of female flowers (true to the genetic sex at the expiry of the effect of treatment), numerous intersexual flowers were observed in the 2 treatments. GA_3 also caused a marked increase in shoot length over controls. Interestingly this was not observed with AgNO_3 . The treated plants were equal to the controls in height.

Discussion. The induction of male flowers by Ag^+ , in the female plants of *Cannabis* reported here, strengthens the concept that endogenous ethylene is probably responsible for

female sex expression in this plant⁸. However, the mode of action of Ag^+ is still unclear. It has been proposed that Ag^+ can act at the receptor site of ethylene attachment, which is believed to contain a metal⁹. The direct action thus envisaged for Ag^+ possibly explains why the response to it is much greater than to GA_3 in inducing maleness in *Cannabis*. Although sex is genetically determined in *Cannabis*, sex-expression is influenced by several factors. As far as hormonal factors are concerned, there is evidence that sex expression is controlled by balance between levels of GA(s) and ethylene – higher ethylene levels favouring femaleness and higher GA levels favouring maleness⁸. When ethylene activity in female plants is blocked by Ag^+ or the relative GA levels are increased by exogenous GA, maleness is induced.

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- 2 E.M. Beyer, Jr, *Pl. Physiol.* 58, 268 (1976).
- 3 R.E. Byers, L.R. Baker, H.M. Sell, R.C. Herner and D.D. Dilley, *Proc. nat. Acad. Sci., USA* 69, 717 (1972).
- 4 J. Rudich, A.G. Halevy and N. Kedar, *Pl. Physiol.* 49, 998 (1972).
- 5 E.M. Beyer, Jr, *Hort Sci.* 11, 195 (1976).
- 6 H.Y. Mohan Ram and V.S. Jaiswal, *Experientia* 26, 214 (1970).
- 7 H.Y. Mohan Ram and V.S. Jaiswal, *Planta* 105, 263 (1972).
- 8 H.Y. Mohan Ram and V.S. Jaiswal, in: *Plant Growth Substances*, p. 987. Hirokawa Publishing Company Inc., Tokyo 1974.
- 9 F.B. Abeles, in: *Ethylene in Plant Biology*, p. 237. Academic Press, New York and London 1973.

A specific GT_1 ganglioside-luteinizing hormone interaction induces conductance changes in lipid bilayers

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Summary. A specific interaction was demonstrated between GT_1 gangliosides incorporated in bilayer membranes and luteinizing hormone. This interaction would allow the penetration of a hormone subunit in the membrane. The results are discussed in terms of adenylate cyclase activation.

Recent studies suggest that gangliosides or ganglioside-like structures may be basic components of glycoprotein hormone receptor¹⁻⁶. These recognition properties of natural cell membranes can be duplicated in model membranes⁷⁻¹². In this report, we present evidence of a specific interaction between luteinizing hormone and GT_1 ganglioside incorporated in a planar bilayer membrane. The conductance change of the lipid bilayer is discussed in terms of the adenylate cyclase activation process.

Materials and methods. Luteinizing hormone (LH), glycerol monooleate (GMO), N-acetylgalactosamine and N-acetylneuraminic acid were purchased from Sigma Chemical Co. GT_1 ganglioside (N-acetylneuraminylgalactosyl-N-acetyl-galactosaminyl-(N-acetylneuraminyl-N-acetylneuraminyl)-galactosylglucosylceramide), GD_{1a} ganglioside (N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosylceramide) and GM_1 ganglioside (galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosylceramide) were Supelco products. Lactose, glucose and galactose were 'pro analysi' products from Union Chimique Belge. N-Decane, a reagent grade product was redistilled before used. The mixtures GMO-gangliosides were dissolved in a chloroform/methanol/deca (30/5/65) mixture and bilayers were formed at room temperature on a 1.3-mm diameter

aperture in a teflon cell separating 2 aqueous phases. Black lipid membrane formation was observed under reflected light with a low power microscope. The aqueous phase contained 0.15 M NaCl + 0.05 M Tris-HCl at pH 7.3. The membrane specific conductance was determined by measuring the specific current I_m/cm^2 as a function of imposed potentials differences V_m , with a 602 Keithley electrometer. The complete system was enclosed in a Faraday cage.

Results and discussion. Conductances of GMO planar bilayer membranes containing GT_1 , GM_1 or GD_{1a} ganglioside were measured before and after addition of LH in the aqueous phase. A 4-fold increase of membrane conduc-

Effect of LH on the conductance of planar membranes containing gangliosides

Bilayers	Conductance $10^{-8} \Omega^{-1} \cdot \text{cm}^{-2}$	
	Without LH	With LH*
GMO	4.6 ± 0.7	5.1 ± 1
GMO- GD_{1a}	6.6 ± 1	8.0 ± 1.4
GMO- GM_1	7.6 ± 1.3	11.0 ± 2.0
GMO- GT_1	16.0 ± 3	60.0 ± 9

* LH concentration 120 $\mu\text{g/ml}$. Molar ratio GMO-ganglioside 97/3.

tance was observed in the presence of GT₁. No significant effect was obtained with GMO, GM₁ ganglioside and GD_{1a} ganglioside (table).

In order to determine whether the LH-GT₁ interaction was specific, experiments were carried out in the presence of an equimolar mixture (100 µg/ml) of the saccharide residues (lactose, galactose, glucose, N-acetylgalactosamine) present in the hydrophilic moiety of the gangliosides. The observed conductance ($15.2 \cdot 10^{-8} \Omega^{-1} \cdot \text{cm}^{-2}$) was nearly identical with the value obtained with the GMO-GT₁ bilayer in absence of LH in the aqueous phase (table). The fact that the saccharide mixture completely reverses the GT₁-LH interaction supports the conclusion that LH interacts specifically with the carbohydrate moiety of the ganglioside.

We observed recently a change in the GMO-GT₁ membrane conductance in the presence of thyrotropin⁷. Similarly, it was demonstrated that a hyperpolarization of thyroid cell membranes can be induced by this hormone on cultured cells via a specific interaction with the thyrotropin receptor¹³. These permeability modifications suppose the penetration of the hormone in the membrane. Fluorescence studies indicated that the LH-GT₁ interaction induces a hormone conformational change³ which would allow the translocation of a hormone subunit in the lipid layer³⁻⁵ inducing the observed conductance changes. These modifications in the lipid organization may be an important step in the sequence of events leading to the adenylate cyclase activation.

- 1 B.R. Mullin, T. Pacuszka, G. Lee, L.D. Kohn, R.O. Brady and P.H. Fishman, *Science* 199, 77 (1978).
- 2 S.M. Aloj, L.D. Kohn, G. Lee and M.F. Meldolesi, *Biochem. biophys. Res. Commun.* 74, 1053 (1977).
- 3 G. Lee, S.M. Aloj and L.D. Kohn, *Biochem. biophys. Res. Commun.* 77, 434 (1977).
- 4 G. Lee, S.M. Aloj, R.O. Brady and L.D. Kohn, *Biochem. biophys. Res. Commun.* 73, 370 (1976).
- 5 B.R. Mullin, P.H. Fishman, G. Lee, S.M. Aloj, F.D. Ledley, R.J. Winand, L.D. Kohn and R.O. Brady, *Proc. natl Acad. Sci. USA* 73, 842 (1976).
- 6 M.F. Meldolesi, P.H. Fishman, S.M. Aloj, F.D. Fedley, G. Lee, R.M. Bradley, R.O. Brady and L.D. Kohn, *Biochem. biophys. Res. Commun.* 75, 581 (1977).
- 7 A. Poss, M. Deleers and J.M. Ruyschaert, *FEBS Lett.* 86, 160 (1978).
- 8 M.T. Tosteson, F. Lau and D.C. Tosteson, *Nature* 243, 112 (1973).
- 9 A. Suroia, B.K. Bachhawat and S.K. Podder, *Nature* 257, 802 (1975).
- 10 R.L. Juliano and D. Stamp, *Nature* 261, 235 (1976).
- 11 J.M. Ruyschaert, A. Tenenbaum, C. Berliner and M. Delmelle, *FEBS Lett.* 81, 406 (1977).
- 12 M. Deleers, A. Poss and J.M. Ruyschaert, *Biochem. biophys. Res. Commun.* 72, 709 (1976).
- 13 E.F. Grollman, G. Lee, F.S. Ambesi-Impimbato, M.F. Meldolesi, S.M. Aloj, H.G. Coon, H.R. Kaback and L.D. Kohn, *Proc. natl Acad. Sci. USA* 74, 2352 (1977).

Phosphomonoesterases in the 2 sexes of the root-knot nematode, *Meloidogyne lucknowica* Singh, 1969

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Summary. The 2 phosphomonoesterases of the root-knot nematode were colorimetrically determined. Alkaline phosphatase activity was observed to be lower than the acid phosphatase activity. Sex related trends were clearly seen in the enzyme levels of the 2 sexes of the nematode. Alkaline phosphatase level differed 28.76%, while acid phosphatase level differed 60.36% in the 2 sexes.

The study of the 2 phosphatases is important, due to their role in transport processes of the nematode². High enzymatic activity has been shown at the luminal and vascular borders of tubular cells of both plants and animals. Both the phosphatases have been studied in several plant parasites, such as *Meloidogyne*³, *Ditylenchus* and *Panagrellus*⁴ and *Meloidogyne* and *Tylenchus*⁵. But the effect of sex has rarely been observed on the enzyme levels of plant nemas.

Materials and methods. 650 brinjal plants (*Solanum melongena*) were collected from Government garden Ali-ganj and other localities of Lucknow, from which 85% were infected with *Meloidogyne lucknowica*. The infected roots were placed in water in 2 petridish and shredded carefully with fine needles. The parasites were kept in 0.7% saline in small cavity blocks. 20% homogenate was prepared in normal saline and kept at 4°C, well-protected from light. The nematodes of 2 sexes were separately collected from the same host plant and processed simultaneously. The females were carefully and completely freed from the gelatinous matrix of the egg capsules. Method of King and Wootton⁶ was followed for the determination of the 2 phosphatases. OD was determined with Bausch and Lomb Spectronic-20 Colorimeter, at 650 µm against blank.

Results and discussion. The normal values of the 2 phosphatases in the 2 sexes of *M. lucknowica* have been given in the table. The males had higher phosphatase activity than their

females. Both the phosphatases have been observed to be present in traces in the cuticle and hypodermis of gelatinous matrix of eggs of *M. javanica*, when examined histochemically⁵. No alkaline phosphatase activity was found in zymograms of *Ditylenchus trifurmis* and *Panagrellus redivivus*⁶. Veech and Endo⁷ observed histochemically greater phosphatase activity at the sites of infection, even in the host soya bean infected with *M. incognita acrita*; thus the

Phosphomonoesterases in the 2 sexes of root-knot nematode, *Meloidogyne lucknowica*

Enzymes	No. of experiments	Sex*	Enzyme activity kA units/g**
Acid phosphatase	12	M	7.14 ± 2.21 (5.00 – 9.06)
	24	F	2.83 ± 1.40 (1.02 – 4.50)
Alkaline phosphatase	12	M	0.73 ± 0.46 (0.12 – 1.02)
	24	F	0.52 ± 0.11 (0.12 – 1.20)

* M: male, F: female. ** Mean ± SD (range in parentheses).