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EFFECT OF PLANT GROWTH SUBSTANCES ON MEMBRANE PERMEABILITY OF UREA AND ERYTHRITOL

PAUL HESTER and WILLIAM STILLWELL

Purdue University School of Science, Biology Department, 1125 East 38th Street, P.O. Box 647, Indianapolis, IN 46223 (U.S.A.)

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Four groups of plant growth substances (the auxins, gibberellins, abscisic acid and cytokinins) were tested as to their effects on increasing the permeability of egg phosphatidylcholine liposomes to erythritol and urea. The cytokinins (kinetin and benzyladenine) produced permeability changes down to about 30 μM for erythritol or urea while the auxins (indoleacetic, indolebutyric and indolepropionic acid), gibberellic acid and abscisic acid affected permeability only at much higher concentrations (above 1300 μM). These results question the physiological significance of the auxins, gibberellic acid and abscisic acid as lipid bilayer perturbing agents.

In 1944 Veldstra [1] proposed growth substances may exert their initial effects by altering membranes. Since then, many often conflicting reports have appeared regarding growth substance-induced membrane permeability changes with various complex biological tissues. These reports have involved the four main classes of plant hormones (the auxins, gibberellins, abscisic acid and the cytokinins).

It has been suggested that auxins exert their initial effects at the membrane level influencing root elongation [2], or perhaps changing water permeability of stems by altering an unknown membrane component [3]. Auxin has also been shown to shift the thermal transition of lipid bilayers [4] and to interact with phosphatidylcholine in deuterochloroform [5,6]. Paleg and co-workers have observed that gibberellic acid affects bilayer permeability to chromate, glucose and sucrose [7], changes membrane fluidity [4], and complexes in deuterochloroform with the charged trimethylamino group of phosphatidylcholine [6,8]. Abscisic acid increases permeability of plant cells to water [9] affecting mesophyll cell structure and altering the uptake of K^+ and Cl^- into coleoptile sections [10,11]. Ion permeability enhancement was proposed to be through the formation of abscisic acid-membrane channels [12]. Kinetin affects the permeability of several types of membranes resulting, for example, in increased permeability to thiourea and urea in onion epidermal cells [13], decreased absorption of phosphate in beet root tissue [14], increased water permeability in radish cotyledons [15], and changes in monovalent cation transport in sunflower cotyledons [16].

The above are but a few of the many diverse examples of the effect of plant growth substances on membranes. Because most of these studies have involved whole cells and organelles or complex natural membrane systems with changes measured over long times, they have been subject to conflicting and uncertain interpretations, and the hormone-phospholipid interactions reported by Paleg and co-workers [5,6,8] were run in a very unbiological environment, deuterochloroform. Due to the almost limitless possible combinations of

phospholipid head groups and tails found in plant membranes interacting with various concentrations of different hormones, the nature of rapid, specific interactions between the growth substances and the membrane at the molecular level remains unresolved. For example, it has not even been clearly demonstrated whether the growth substances interact preferentially with a protein or lipid component of the membrane.

Utilizing a protein-free artificial bilayer system in place of a natural membrane, several workers have attempted to better define a growth substance-bilayer interaction. The report of Wood and Paleg [7] on gibberellic acid-induced enhancement of bilayer permeability was augmented by Pauls et al. [17], who showed the growth substance decreased the phase transition of synthetic phosphatidylcholines and phosphatidylglycerol. Lelkes et al. [18] reported a similar auxin-induced decrease in the phase transition of dimyristoylphosphatidylcholine bilayers. Using electrical techniques with planar bimolecular lipid membranes, Lea and Collins [12] proposed that abscisic acid can induce membrane channels. We reported the effect of kinetin on water and glucose permeability across bilayers composed of several types of phospholipids including the natural phospholipids egg phosphatidylcholine and asolectin and the synthetic phosphatidylcholines, dimyristoyl- and dipalmitoylphosphatidylcholine [19]. These results demonstrated that kinetin acts on the lipid bilayer as a general membrane perturbing agent, affecting not only water permeability, but solute permeability in general. Although the artificial membrane studies imply that at high enough concentration the four classes of growth substances may affect the lipid bilayer component of membranes, they all employ unphysiologically high levels of the hormones.

Here we compare the relative effects of four classes of growth substances (the auxins, gibberellic acid, abscisic acid and cytokinins) on the permeability to the non-charged solutes erythritol and urea. Erythritol and urea are chosen because spectrophotometric methods have been developed for measuring their rapid permeability with liposomes.

Egg phosphatidylcholine liposomes (Sigma Type 1X-E) were made by the method of Bangham [20] in 40 mM glucose, 10 mM Tris-HCl, pH 7.5. Following centrifugation to remove non-liposomal lipids, the liposomes were re-incubated for 30 min with appropriate amounts of growth substance. Kinetin, abscisic acid and gibberellic acid were purchased from Sigma and benzyladenine, indoleacetic, indolebutyric and indolepropionic acid from ICN. Rapid time swelling was followed in isotonic (40 mM) erythritol or urea containing 10 mM Tris-HCl by the method of De Gier and co-workers [21,22]. Small changes in absorbances were measured on a Beckman DU-8 Computing Spectrophotometer at 350 nm, temperature controlled at 25 ± 0.1°C.

Fig. 1 illustrates erythritol permeability rates (swelling of egg phosphatidylcholine liposomes in isotonic erythritol) as a function of increasing concentrations of plant growth substances. The auxins (indoleacetic, indolebutyric and indolepropionic acid), as well as gibberellic acid and abscisic acid, affect membrane permeability to erythritol, but only at relatively high concentrations ranging from 1300 μM for indolebutyric acid to 13000 μM for gibberellic acid. Kinetin and benzyladenine induced similar permeability changes but at concentrations of only 30 μM.

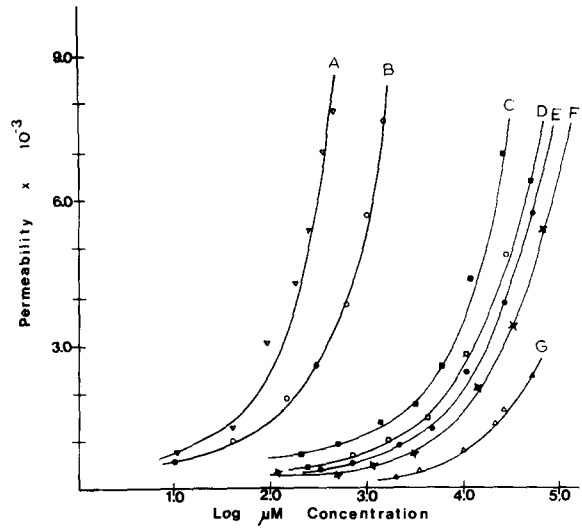

Fig. 1. The effect of auxins (indoleacetic, indolebutyric, indolepropionic acid), gibberellic acid, abscisic acid and the cytokinins (kinetin and benzyladenine) on the permeability of erythritol to egg phosphatidylcholine liposomes. A, benzyladenine; B, kinetin; C, indolebutyric acid; D, indolepropionic acid; E, indoleacetic acid; F, abscisic acid; G, gibberellic acid.

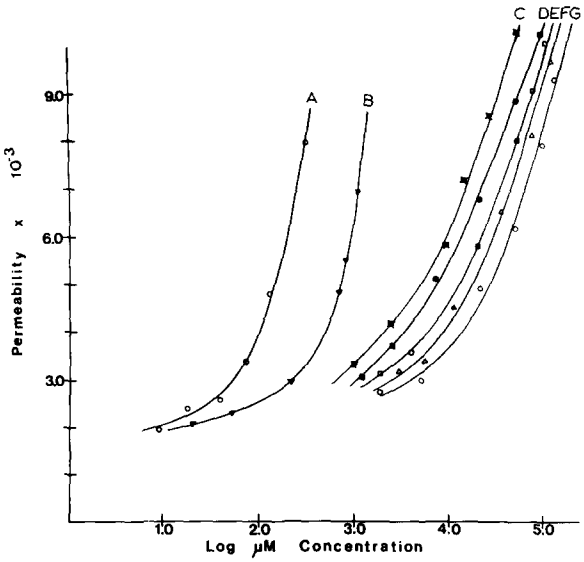

Fig. 2. The effects of auxins (indoleacetic, indolebutyric, indolepropionic acid), gibberellic acid, abscisic acid and the cytokinins (kinetin and benzyladenine) on the permeability of urea to egg phosphatidylcholine liposomes. A, kinetin; B, benzyladenine; C, abscisic acid; D, indolebutyric acid; E, indolepropionic acid; F, gibberellic acid; G, indoleacetic acid.

In Fig. 2, kinetin and benzyladenine are shown to enhance urea permeability at concentrations of 5 to 430 times less than the other growth substances. From both figures, it is obvious that the cytokinins are much more effective at enhancing bilayer membrane permeability than are the auxins, gibberellic acid or abscisic acid.

Previous experiments, linking the function of plant growth substances to natural bilayer membranes, have raised as many questions as they have answered. Here we use the simple, protein-free liposome system to investigate possible interactions of the growth substance with just the lipid bilayer component of the membrane. Although all of the tested growth substances enhanced membrane permeability, there is a marked difference between the effect produced by kinetin and benzyladenine and that produced by the other growth substances. These results indicate that of the substances tested, only the cytokinins actually affect the bilayer at low levels.

Although this report by no means proves kinetin exerts its physiological effect at the lipid bilayer of membranes, it does raise the question as to the possible significant effect on bilayers of auxins, gibberellic acid or abscisic acid, which were required in much higher levels than kinetin. Perhaps these growth substances do affect natural membranes but in an indirect manner or else may interact with a specific, but as yet unknown, membrane protein or membrane lipid. It is also possible that the initial site of action for these compounds is other than at the membrane surface. This report further substantiates the possible direct role of kinetin and benzyladenine as general membrane perturbing agents. Further experiments measuring rapid membrane permeability changes associated with specific plant hormone-phospholipid interactions are in progress.

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