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THE EFFECT OF INDOLEACETIC ACID ON PHOSPHOLIPID METABOLISM IN PEA STEMS*

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Key Word Index—*Pisum sativum*; Leguminosae; stem sections; indoleacetic acid; phospholipid metabolism; phosphatidylcholine.

Abstract—Indole-3-acetic acid (IAA) was found to stimulate stem elongation but inhibit the incorporation of [^{14}C]choline into phosphatidylcholine within 1 hr of treatment of *Pisum sativum* cv Feltham First stem sections. Abscisic acid reversed these effects. No effect of IAA was detected on [^{14}C]ethanolamine incorporation into phosphatidylethanolamine or of [^{14}C]acetate incorporation into lipids. The amount of phosphatidylcholine on a fresh weight basis was decreased and the relative levels of its component fatty acids were also changed by IAA treatment of stem sections.

INTRODUCTION

Several plant growth regulators have been shown to interact with the membranes of various tissues. For example, auxins bind with high affinity to the rough endoplasmic reticulum of maize coleoptiles [1], ethylene affects secretion [2] and gibberellins can stimulate membrane proliferation [3, 4] as well as interact directly with phosphatidylcholine in membranes [5]. In spite of the possible implications of hormone–membrane interactions for membrane lipid metabolism, there have been few reports concerning the effects of plant growth regulators on phospholipid metabolism. The major exception is the gibberellins, which have been reported to stimulate phospholipid synthesis in barley aleurone tissue [6–8] and indeed to stimulate specific enzyme activities [7]. However, these interpretations have been questioned [6] and, in wheat aleurone, similar results could not be obtained [9–11]. In a recent study it was found that gibberellic acid caused a decrease in the total amounts of aleurone phosphatidylcholine by increasing its rate of degradation [12].

Since information comparable to that for gibberellins is not available for auxins, we chose to investigate the effects of indole-3-acetic acid (IAA) on the synthesis of the major phospholipids of an auxin-responding system. We decided to use the third internode of post-germination pea seedlings, since auxin effects on such tissue have been well characterized [13], they would be rich in phospholipids, particularly phosphatidylcholine and phosphatidylethanolamine [14, 15], and some information was available on phospholipid synthesis in this tissue [16].

RESULTS

Elongation

IAA stimulated growth in the excised pea stem segments at concentrations as low as 0.3 μM (Fig. 1A). With 5 μM IAA, the stimulation was apparent within 1 hr following its addition and continued to 6–7 hr following addition of the hormone (Fig. 1B). The hormone was not added until after 1 hr of incubation in order to avoid complications arising from the early burst of growth following excision of the tissue.

In the absence of IAA, little growth occurred, so that by comparison the IAA-stimulated growth was considerable. For example, at 2 hr after addition of the hormone, the controls had increased about 0.1 mm in length, while the IAA-treated material increased about 0.4 mm (from the time of hormone addition)—a 4-fold stimulation. In both cases, however, the increase compared to the total length was very small; in the presence of IAA this was 5% but in its absence 1%. This was probably due to the dwarf variety of pea used. The particular variety, Feltham First, was employed because considerable information was available about its lipid metabolism (e.g. [17]) and because we have found that results obtained with it are very reproducible. Indeed, the IAA-stimulated elongation was highly reproducible and always significant at least at the 0.05% level in any single experiment.

Abscisic acid (ABA) alone had little effect on linear growth of the stem segments but, when added with IAA, it prevented IAA-stimulated elongation (Fig. 2). This inhibition was apparent after both 1 and 5 hr of treatment.

Choline incorporation

In most experiments IAA had little effect on the uptake of radioactive choline into the pea-stem sections, nevertheless it decreased the incorporation into the lipid fraction within 1 hr (Table 1). These effects continued for up to 3 hr, but were reduced by 5 hr after the beginning of the

*Part 1 in the series "Phospholipid Metabolism in Pea (*Pisum sativum*) stems".

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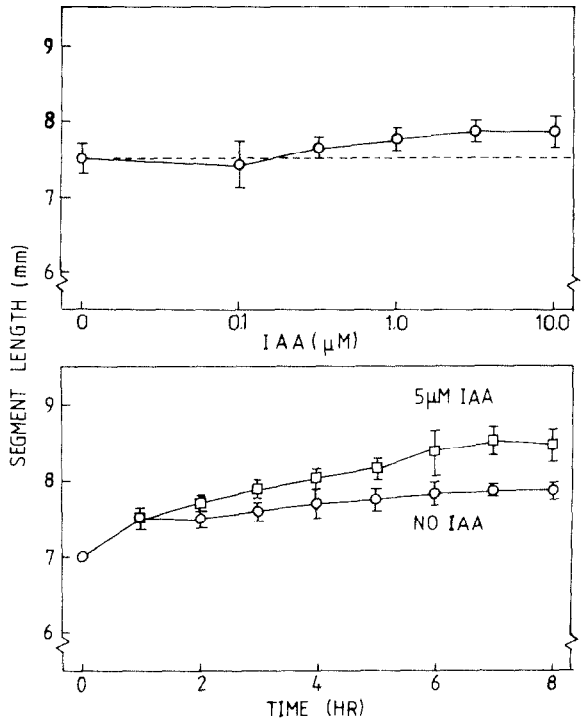

Fig. 1. Elongation response of stem sections from the dwarf pea variety Feltham First to indoleacetic acid. In (A) sections were exposed to various IAA concentrations for 2 hr. In (B) sections were exposed to 5 μM IAA for various times. The sections were prepared, exposed to IAA and measured as described in Experimental. Bars represent standard errors for triplicate determinations with 10 stems/replicate.

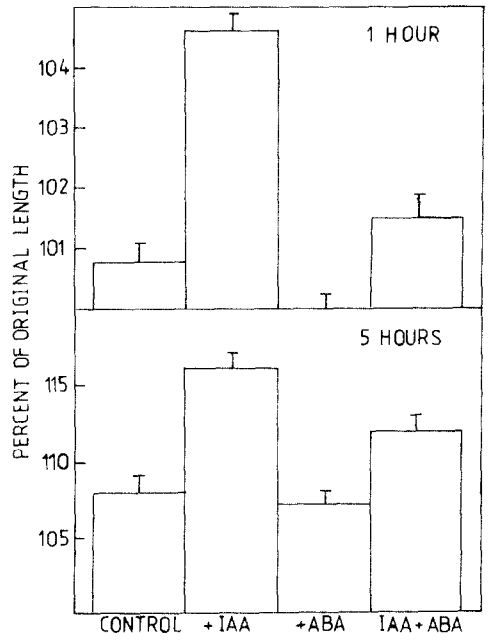

Fig. 2. The effects of indoleacetic acid and abscisic acid on elongation of pea-stem sections. The experiments were carried out as described in Experimental for 1 or 5 hr. The bars represent standard errors for triplicate determinations with 10 stem sections/replicate.

treatment (Table 2). The only significant [^{14}C]choline-labelled component in the lipids separated by TLC was phosphatidylcholine; this compound accounted for *ca* 97.5% of the radiolabelled lipids. The labelling of this phospholipid from [^{14}C]choline was very significantly reduced by IAA treatment of stem sections (Table 1). Although the mean inhibition of this labelling was *ca* 27%, in individual experiments it could be as much as 45% – greater inhibition occurring in stem sections whose elongation was stimulated by IAA to a greater extent. At incubation times of greater than 5 hr, IAA stimulated the incorporation of [^{14}C]choline into phosphatidylcholine (results not shown).

ABA alone had no significant effect on choline uptake into stem sections, or incorporation into the total lipid and phosphatidylcholine fractions. However, it again reversed the effect of IAA (Table 3).

Ethanolamine and acetate incorporation

No significant effect of IAA on the incorporation of ethanolamine into lipids was found, although a small

Table 1. The effect of IAA on [^{14}C]choline uptake into pea-stem sections and incorporation into lipids

	Uptake (% added counts)	$CHCl_3$-soluble counts (% uptake)	PC labelling (% uptake)	Stem length (mm)
Control	1.24 ±0.13(11)	7.53 ±0.71(11)	3.73 ±0.54(11)	7.238 ±0.023(12)
$+10^{-5}$ M IAA	1.34 ±0.11(11)	5.86* ±0.46(11)	2.64** ±0.27(11)	7.501*** ±0.049(12)

Experiments were carried out as described in Experimental with incubations for 1 hr. Results are expressed as means ±S.E.M.s with the number of independent experiments indicated in parentheses. The data were tested for significance by Student's *t*-test for paired samples; * = $P < 0.02$, ** $P < 0.005$, *** $P < 0.001$. Abbreviations: IAA = indole acetic acid; PC = phosphatidylcholine.

Table 2. The effect of IAA on [^{14}C]choline uptake into pea-stem sections and incorporation into lipids as a function of time

Time (hr)	Total uptake (% control)		Total $CHCl_3$-soluble counts (% control uptake)	
	−IAA	+IAA	−IAA	+IAA
1	100	127 ± 10	6.9 ± 0.7	5.8 ± 0.9
3	100	95 ± 3	9.6 ± 1.1	6.8 ± 1.5
5	100	109 ± 2	9.0 ± 0.3	8.5 ± 1.4

IAA was used at 10^{-5} M. Treatments were in triplicate with 10 sections/replicate. Values are expressed as means ± S.E.M.

stimulation of uptake into the stem segments was obtained (data not shown). Acetate uptake into the tissue was increased in the presence of IAA for 1 hr, but decreased after 3 hr. In contrast, there was no difference in the incorporation of radiolabel from [^{14}C]acetate into the major phospholipids, phosphatidylcholine, phosphatidylethanolamine or phosphatidylinositol.

Lipid composition

The lipid composition of control and IAA-treated tissue is shown in Table 4. In the untreated controls, the major phospholipid was phosphatidylcholine although phosphatidylethanolamine and phosphatidylinositol were also present in similar amounts. In IAA-treated tissue, there was a large decrease in the absolute level of phosphatidylcholine per g fresh weight of tissue so that phosphatidylethanolamine became the most prevalent lipid. Phosphatidylinositol was also reduced but there were no changes in the other acyl lipid fractions.

Despite the absence of an effect on acetate incorporation into lipids, treatment with IAA caused distinct shifts in the fatty acid composition of certain lipid classes (Table 4). For example, the relative amounts of palmitate and oleate in phosphatidylinositol changed dramatically, with the saturated fatty acid being considerably increased upon IAA treatment. Within phosphatidylcholine there was a large decrease in oleate and an increase in linolenate. Additional but smaller shifts also occurred in other lipid fractions. Some of these changes may have been caused by the decrease in the levels of certain molecular species of particular lipids. However, because of the size of the alterations, there must have been considerable redistribution or modification of fatty acids within lipids.

DISCUSSION

IAA has a rapid effect on phospholipid metabolism in the pea-stem segments and the effect appears to be rather specific. The inhibition of choline incorporation into

Table 3. The effects of abscisic acid and indole acetic acid on [^{14}C]choline uptake into pea-stem sections and incorporation into lipids

Expt. No.	Total uptake (% control)				Total chloroform-soluble counts (% control uptake)				Phosphatidylcholine (% control uptake)			
	Control	+IAA	+ABA	+ABA +IAA	Control	+IAA	+ABA	+IAA +ABA	Control	+IAA	+ABA	+ABA +IAA
1	100	135	116	136	7.0	5.0	7.0	9.3	2.8	2.5	3.2	4.8
2	100	108	145	125	8.4	8.1	8.3	8.6	4.0	2.5	3.1	3.2
Mean ± S.E.M.	100	122 ± 14	130 ± 14	131 ± 5	7.7 ± 0.7	6.6 ± 1.5	7.7 ± 0.7	8.9 ± 0.4	3.4 ± 0.6	2.5 ± 0.0	3.2 ± 0.1	4.0 ± 0.8

IAA and ABA (abscisic acid) were used at 10^{-5} M and the experiments carried out in triplicate with 10 stem sections/replicate as described in Experimental. Incubations were for 1 hr.

Table 4. Acyl lipid composition of untreated and indoleacetic acid-treated pea stems

Lipid	Control Fatty acids (% total)							IAA-treated Fatty acids (% total)						
	Quantity (μg)	16:0	18:0	18:1	18:2	18:3	Other	Quantity (μg)	16:0	18:0	18:1	18:2	18:3	Other
Phosphatidylinositol	210	29	8	19	35	5	4	179	49	3	1	41	2	4
Phosphatidylcholine	250	16	8	12	51	12	1	187	17	3	2	50	28	tr.
Phosphatidylglycerol	60	30	tr.	tr.	40	30	tr.	59	11	n.d.	6	50	27	6
Phosphatidylethanolamine	207	28	1	4	53	12	2	198	30	4	7	43	14	2
Neutrals	89	15	1	12	40	24	8	80	24	5	14	23	23	11

The data represent the average of 3 determinations. IAA treatment was with 10^{-5} M solutions for 1 hr. The quantities of acyl lipids and their fatty acid patterns were quantitated from GC data (see Experimental). Average fresh weights of tissues used were 250 mg for controls and 252 mg for IAA-treated. Abbreviations: tr. = trace (< 0.5); n.d. = none detected. Fatty acids are expressed with the number before the colon representing the chain length and the number afterwards the number of double bonds.

phosphatidylcholine but the absence of a similar effect on ethanolamine incorporation, along with the decrease in phosphatidylcholine levels and the apparent redistribution of fatty acids in the various lipids, all argue in favour of this specificity. It is, perhaps, of interest that in barley tissues, where an increase in phosphatidylcholine labelling from [^{14}C]choline by gibberellin treatment has been reported [4], labelling from [^{14}C]acetate was also unaffected [6, 8]. In addition, Mirbahar and Laidman [12] in their studies with wheat found that phosphatidylcholine was the only phospholipid whose absolute level was reduced by gibberellic acid treatment.

The significance of the events reported here cannot be determined from these data. The speed of the effect of IAA on choline incorporation supports the possibility that this effect is associated with the elongation occurring at the same time. The reversal of both elongation and the effect on phosphatidylcholine labelling by ABA also supports a relationship between the two events. Whether the IAA effects on choline incorporation are related to the cause of elongation, are a part of the metabolism associated with the elongation, are a consequence of elongation, or are unassociated cannot be stated at present.

Although the effect of IAA on phosphatidylcholine levels and metabolism appear small, they may indeed be major at the cellular level. Pea-stem sections have at least a dual compartmentation of phosphatidylcholine synthesis. Activity has been found in both the endoplasmic reticulum and the Golgi apparatus [16]. The small overall effect would become large if it were on only one of these organelle systems.

The rapid effect of IAA on phosphatidylcholine labelling from [^{14}C]choline also raises questions as to the enzymic mechanism responsible. The lack of labelling of phosphatidylcholine from [^{14}C]ethanolamine in the present work indicates that, as with other plant tissues [18, 19], the phospholipid is mainly made *de novo* by the CDP-base pathway. In animal tissues it is generally considered that cytidylyl transferase is the rate-limiting enzyme of this pathway and one which is also subject to hormonal control [20]. This enzyme, as well as the CDP-choline: diacylglycerol phosphocholine transferase, was increased in barley aleurone tissue by gibberellin treatment [7]. However, in leaf tissue, gibberellic acid caused a decrease in the levels of choline kinase [21]. It would be interesting to see which enzyme is regulatory in the pea-stem sections, particularly in view of the rapid effect which IAA has on phosphatidylcholine in such tissues.

EXPERIMENTAL

Materials. Pea (*Pisum sativum* cv Feltham First) seeds were purchased from Butcher's Seeds, Croydon, Surrey, U.K. [1-^{14}C]Acetate (sp. radioactivity 2.0 GBq/mmol), [2-^{14}C]ethanolamine (sp. radioactivity 2.0 GBq/nmol), [methyl-^{14}C]choline (sp. radioactivity 2.15 GBq/nmol) and PCS scintillant solution were from Amersham International. IAA, ABA and 8-anilino-1-naphthalene sulphonic acid were from Sigma. Fatty acid standards were from Nu-Chek, Elysian, MN, U.S.A. Other chemicals were of the best available grade and were from B.D.H.

Incubations. Pea seeds were sown in vermiculite and grown for 10 days at 20° with *ca* 2 klx illumination. For expts, the tissue was harvested and 7 mm long sections from the third internode were excised under 20 mM KPi buffer, pH 6.5. These sections were placed into a beaker with adequate buffer to wet, but not submerge, the sections. The beaker was covered and the sections incubated in the dark for 1 hr at 20° in order to allow for an initial burst of growth presumably due to handling and cutting the tissue (Fig. 1).

After the initial 1 hr period, stem segments were measured as required by gently removing them from the beaker, blotting briefly and laying them against a metric rule to estimate their length. The sections were then incubated in 50 ml beakers containing 2 ml of a 20 mM KPi buffer, pH 6.5 [13]. Growth regulator(s) and radioisotope were added as required. The concns of IAA and ABA were 10 μM unless otherwise indicated. [Methyl-^{14}C]choline chloride, [2-^{14}C]ethan-1-ol-2-amine and [1-^{14}C]acetate were all added at 0.5 μCi, except when 4 μCi of acetate was used for the fatty acid analysis experiments. Five stem sections were usually incubated per beaker and each experiment was carried out at least in triplicate. At the end of the incubation, stem sections were measured as described above. The time of incubation was as described in the Results.

Extraction and analysis of lipid. At the end of the incubation period, tissue samples were briefly rinsed in a 10 mM buffered soln of the unlabelled precursor (choline, ethanolamine or acetate). The sections were then transferred to 1 ml of propan-2-ol in screw-top tubes and heated at 70° for 20 min to stop the reaction. After cooling, the segments were homogenized in propan-2-ol using a mortar and pestle, and the extraction was completed by the method of ref. [22] with propan-2-ol replacing MeOH in the procedure.

Total lipids were separated by TLC on silica gel G as described in ref. [23]. Lipid bands were revealed by spraying the plates with a 0.2% (w/v) soln of 8-anilino-1-naphthalene sulphonic acid in MeOH and viewing under UV light. The lipid bands were identified by co-chromatography with authentic standards and the use of specific spray reagents. For a full analysis of pea lipids see refs. [17, 24]. Radioactivity was detected by photography of the TLC plates with a spark-chamber autoradiograph and was estimated by scraping the bands into vials containing PCS–xylene (2:1). Quench correction was by the external standard channels ratio method.

For the determination of fatty acid composition, the lipid bands were scraped into screw-top tubes and methylated with 2.5% (v/v) H_2SO_4 in MeOH and separated by GC on 15% (w/w) EGSS-X on Chromosorb W AW (80–100 mesh). Quantification of fatty acid peaks was with an int. standard of Me pentadecanoate and identification was as described in ref. [17]. Radioactivity was estimated with a gas-flow proportional counter.

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REFERENCES

1. Ray, P. M. (1977) *Plant Physiol.* **59**, 594.
2. Abeles, F. B. (1972) *Annu. Rev. Plant. Physiol.* **23**, 259.
3. Ben-Tal, Y. and Varner, J. E. (1974) *Plant Physiol.* **54**, 813.
4. Evins, W. H. and Varner, J. E. (1971) *Proc. Natl. Acad. Sci., U.S.A.* **68**, 1631.
5. Wood, A., Paleg, L. G. and Spotswood, T. M. (1974) *Aust. J. Plant Physiol.* **1**, 167.
6. Firn, R. D. and Kende, H. (1974) *Plant Physiol.* **54**, 911.
7. Johnson, K. D. and Kende, H. (1971) *Proc. Natl. Acad. Sci., U.S.A.* **68**, 2674.
8. Koehler, D. E. and Varner, J. E. (1973) *Plant Physiol.* **52**, 208.
9. Laidman, D. L., Colborne, A. J., Dolg, R. I. and Varty, K.

(1974) in *Mechanisms of Regulation of Plant Growth* (Bielski, R. L., Ferguson, A. R. and Cresswell, M. M., eds.), pp. 581–590. Royal Soc. New Zealand Bull. Vol. 12.
10. Tomos, A. D. and Laidman, D. L. (1979) in *Recent Advances in the Biochemistry of Cereals* (Laidman, D. L. and Wyn Jones, R. G., eds.), pp. 119–146. Academic Press, London.
11. Varty, K. and Laidman, D. L. (1976) *J. Exp. Botany* **27**, 745.
12. Mirbahar, R. B. and Laidman, D. L. (1982) *Biochem. J.* **208**, 93.
13. Evans, M. L. and Ray, P. M. (1973) *Plant Physiol.* **52**, 186.
14. Harwood, J. L. (1980) in *The Biochemistry of Plants* (Stumpf, P. K. and Conn, E. E., eds.), Vol. 4, pp. 1–55. Academic Press, New York.
15. Hitchcock, C. and Nichols, B. W. (1971) *Plant Lipid Biochemistry*. Academic Press, London.
16. Montague, M. J. and Ray, P. M. (1977) *Plant Physiol.* **59**, 225.
17. Bolton, P. and Harwood, J. L. (1977) *Biochem. J.* **168**, 261.
18. Harwood, J. L. (1979) *Prog. Lipid Res.* **18**, 55.
19. Moore, T. S. (1982) *Annu. Rev. Plant Physiol.* **33**, 235.
20. Vance, D. E. and Choy, P. C. (1979) *Trends Biochem. Sci.* **4**, 145.
21. Tanaka, K. and Tolbert, N. E. (1966) *Plant Physiol.* **41**, 313.
22. Garbus, J., de Luca, H. F., Loomans, M. E. and Strong, F. M. (1963) *J. Biol. Chem.* **238**, 59.
23. Harwood, J. L. and James, A. T. (1975) *Eur. J. Biochem.* **50**, 525.
24. Harwood, J. L. and Stumpf, P. K. (1970) *Plant Physiol.* **46**, 500.