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Inhibitors of animal phospholipase A_2 enzymes are selective inhibitors of auxin-dependent growth. Implications for auxin-induced signal transduction

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Abstract. Auxin and elicitors reportedly activate phospolipase A. A number of inhibitors known to inhibit animal phospholipase A_2 were tested for their ability to inhibit hormone and fusicoccin-induced growth. To this end, growth induced by indolyl-3-acetic acid and 2,4-dichlorophenoxyacetic acid in hypocotyl segments of etiolated zucchini (*Cucurbita pepo* L.) seedlings was determined in the presence of the inhibitors nordihydroguajaretic acid (NDGA), aristolochic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), PB_x (a prostaglandin derivative), and oleylethyl phosphocholine. Each chemical proved inhibitory to auxin-induced growth, oleylethyl phosphocholine being the least effective. The effects of the first three inhibitors were investigated in more detail. Growth induced by 10 μM 2,4-dichlorophenoxyacetic acid or 1 μM indolyl-3-acetic acid was inhibited 50% by about 30–50 μM NDGA, by about 25 μM aristolochic acid, and by about 10–20 μM EYTA. Growth inhibition was reversible and became apparent 0.5–1 h after inhibitor addition. Growth induced by 0.5 or 1 μM fusicoccin was much less inhibited by NDGA and by ETYA, whereas aristolochic acid was only slightly less effective on fusicoccin-induced than on auxin-induced growth. These three inhibitors were also tested for their effects on gibberellin-induced growth in light-grown peas (*Pisum sativum* L.) and on cytokinin-induced expansion growth in excised cotyledons from radish (*Raphanus sativum* L.) seedlings. In both tests, aristolochic acid had toxic side-effects although gibberellin-induced growth was still apparent. In the gibberellin test, neither NDGA at up to 100 μM nor ETYA at 80 μM was inhibitory to hormone-induced growth. Moreover, 40 μM ETYA was not inhibitory to kinetin-induced growth. We hypothesize that the selectivity of phospholipase A_2 inhibitors for auxin-induced growth implies a different signal transduction pathway for each of the different signal substances tested, and that auxins might use fatty acid(s) and/or lysophospholipid(s) or their derivatives as the preferred second messengers.

Key words: Auxin – Auxin inhibitor – *Cucurbita* – Elongation growth – Phospholipase A inhibitor – Signal transduction

Introduction

Phospholipase A (PLA) activity in suspension-cultured soybean cells is rapidly activated within less than 5 min of auxin application (Scherer and André 1989). In hypocotyl segments of sunflower and zucchini this response is also rapid but it is only significantly different from the controls after 15–30 min (Scherer 1995). In isolated membranes, activation of PLA activity is equally rapid and receptor-mediated (Scherer and André 1989; André and Scherer 1991; Scherer and André 1993). Moreover, activation of PLA activity is a receptor-mediated response also to certain elicitors (Farmer and Ryan 1992; Lee et al. 1992; Mueller et al. 1993; Roy et al. 1995; Chandra et al. 1996). This suggests that PLA activation by auxin or other signal molecules could be a typical signal transduction reaction, as is well-known in animal cells (Burch et al. 1986; Jelsema 1987).

In animal systems, activation of a cytosolic PLA (PLA_2) is receptor-mediated and generates arachidonic acid and lysophospholipids as well-established lipid second messengers (Exton 1994). Animal cytosolic PLA_2 is inhibited by a number of structurally unrelated compounds. Some of these inhibitors have also been shown to inhibit agonist-induced responses involving the animal cytosolic PLA_2 (Gerrard 1985; Vishnawath

This paper is dedicated to Professor A. Sievers on the occasion of his retirement

Abbreviations: 2,4-D = 2,4-dichlorophenoxyacetic acid; ETYA = 5,8,11,14-eicosatetraynoic acid; GA_3 = gibberellic acid; NDGA = nordihydroguajaretic acid; OEPC = oleyloxyethyl phosphocholine; PLA (PLA2) = phospholipase A (A_2)

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et al. 1988; Magolda and Galbraith 1989; Ondrey et al. 1989; Hannigan and Williamson 1991; Ponzoni et al. 1992; Rosenthal et al. 1992; Sa and Fox 1994). In order to test our working hypothesis that PLA activation could have a similar role in both plant and animal signal transduction it seemed appropriate to test the inhibitory capacity of these PLA_2 inhibitors in a well-known classical auxin biotest, the growth test, using cut segments of etiolated tissue which also grow in response to the fungal toxin fusicoccin (Marrè et al. 1973; Yamagata and Masuda 1975). Since the possibility that these inhibitors have side-effects in plants cannot be easily ruled out we also tested them in two other hormone-specific biotests, stem growth induced by gibberellic acid (GA_3) in intact light-grown and green pea stems (Kende and Lang 1964) and expansion growth induced by cytokinins in isolated radish cotyledons (Letham 1971).

Materials and methods

Chemicals. All plant hormones were purchased from Sigma (Deisenhofen, Germany). The inhibitors oleyloxyethyl phosphocholine (OEPC) and 5,8,11,14-eicosatetraynoic acid (ETYA) were from Calbiochem (Giessen, Germany), aristolochic acid and nordihydroguajaretic acid (NDGA) from Sigma.

Auxin-induced growth. Zucchini (*Cucurbita pepo* L. cv. Cocozelle von Tripolis; Schmitz-Laux, Hilden, Germany) seedlings were grown for 4 d at 28 °C in the dark. Segments of 1 cm were cut just below the hook and placed in a petri dish with distilled water. Then cuticles were abraded with very fine SiC powder used for polishing (2.5 g powder in 10 mL distilled water) for 20 min on a rotatory shaker at 300 rpm at room temperature (about 25 °C). Stock solutions for auxins and inhibitors were made up in acetonitrile or ethanol at 50 mM or 100 mM so that the solvent content of the assays did not exceed 20–30 µL per 10 mL in a single incubation in 10 mM KH_2PO_4 (pH 5.6). Assays to be compared in individual experiments contained the same amount of solvent, and there was no observable influence of the solvent on extension growth at these concentrations. In each assay, ten hypocotyl segments were measured at a time, lined up with a ruler. In those experiments where duplicates were done the bars indicate the experimental errors ($n = 2$); in most experiments, single assays were run but all experiments were repeated several times.

Gibberellin-induced growth. Dry peas (*Pisum sativum* L. cv. Kleine Rheinländerin; Schmitz-Laux) were soaked overnight in 10 mM KH_2PO_4 at pH 5.6, the buffer just covering the peas in order to avoid anoxia. For experimental treatments, batches of 35 or 50 peas were lightly covered with 50 mL of the same buffer containing hormone and inhibitors at various concentrations, and gently shaken for 6 h on a rotatory shaker (50 rpm). Peas were planted in sterilized earth and grown in the greenhouse under natural light conditions (spring time). Epicotyl lengths were determined with a ruler after 9–15 d.

Cytokinin-induced growth. Radish (*Raphanus sativum* L. cv. Ostergruß rosa2; Nebelung, Münster, Germany) seeds were soaked for 35–40 h in tap water in the dark. Young seedlings with a split seed coat were taken and the cotyledons cut off at the base. The larger of each pair of cotyledons was dried by blotting on filter paper and, in batches of ten, the fresh weight was determined. The starting fresh weights for such batches were kept similar at 50 ± 5 mg. Batches of ten cotyledons were placed in small petri dishes on thick filter papers soaked with 10 mM KH_2PO_4 (pH 5.6) containing various concentrations of the hormone and/or inhibitor. They were kept at room temperature for up to 6 d under dim light and the fresh weight was then determined (Letham 1971).

Results

Several known inhibitors of PLA_2 activity were tested for their effects on auxin-induced growth of hypocotyl segments from etiolated zucchini seedlings. Nordihydroguaiaretic acid (NDGA) inhibited auxin-induced growth at 25–75 µM in the presence of either 10 µM IAA, a natural auxin (Fig. 1A), or 1 µM 2,4-D, an artificial auxin (Fig. 1B). The growth of control hypocotyls was also inhibited by NDGA but the extent of its effect in the absence of added auxin was less than its effect on auxin-induced growth (Fig. 1B). At a 2,4-D concentration of 1 µM the inhibitory effect of 50 µM NDGA was much more pronounced than at 10 µM 2,4-D (Fig. 1C).

The reversibility of inhibition by NDGA was tested. To this end, the recovery from treatments of inhibitor plus 2,4-D was checked by transferring these assays to 2,4-D alone at various times after the start of incubation (0 h). Another comparison was made to assays in which 2,4-D was added alone at 0 h or after 1 h (Fig. 2A).

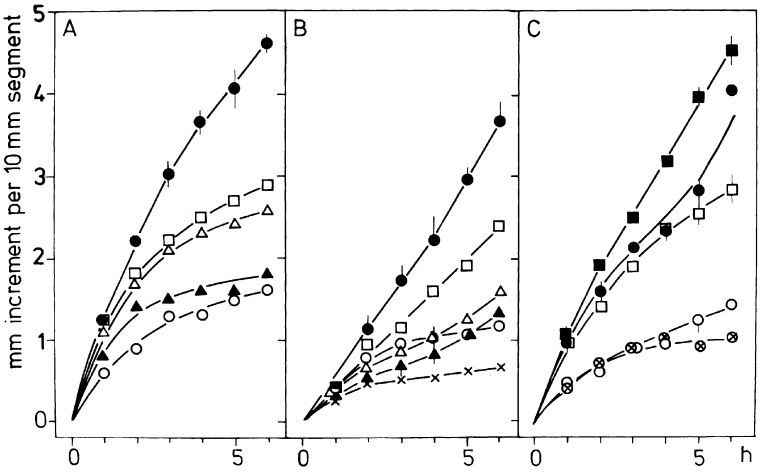

Fig. 1A–C. Influence of NDGA on auxin-induced extension growth of zucchini hypocotyls. **A** IAA at 10 µM in combination with various NDGA concentrations. ○, No addition; ●, 10 µM IAA (triplicate assays; SD); □, 10 µM IAA + 33 µM NDGA; △, 10 µM IAA + 50 µM NDGA; ▲, 10 µM IAA + 75 µM NDGA. **B** 2,4-D at 1 µM in combination with various NDGA concentrations. ○, No addition; ×, 75 µM NDGA alone; ●, 1 µM 2,4-D alone; □, 1 µM 2,4-D + 25 µM NDGA; △, 1 µM 2,4-D + 50 µM NDGA; ▲, 1 µM 2,4-D + 75 µM NDGA. All assays were done in duplicate. Where the experimental error exceeds the size of the symbols, this is indicated by a bar. **C** Different 2,4-D concentrations in combination with 50 µM NDGA. ⊗, No addition; ■, 10 µM 2,4-D alone; □, 10 µM 2,4-D + 50 µM NDGA; ●, 1 µM 2,4-D alone; ○, 1 µM 2,4-D + 50 µM NDGA. All assays were done in duplicate. Bars indicate experimental error ($n = 2$) whenever symbol size is exceeded

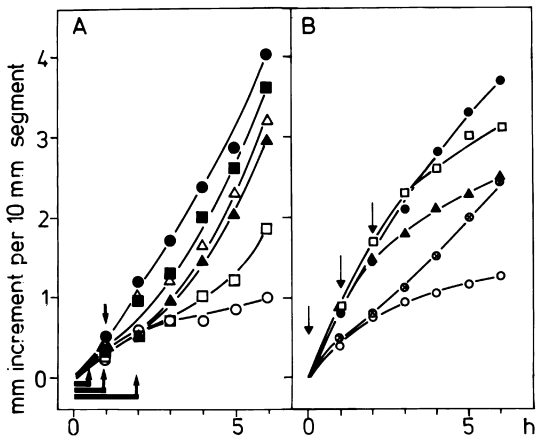

Fig. 2A–B. Reversibility of the inhibitory effect of NDGA on auxin-induced growth of zucchini hypocotyls. **A** 2,4-D at 1 μM was added alone (●) or in combination with 50 μM NDGA at the start of incubation (0 h). After the times indicated (bar and arrow), 2,4-D + NDGA was replaced by 2,4-D alone: ■, at 0.5 h; ▲, at 1 h; □, at 2 h. For comparison, 2,4-D alone was added 1 h after the start of incubation (*downward pointing arrow*, Δ) or no addition was made (○). Growth recovered at slightly more than 1 h after NDGA withdrawal. **B** Pretreatment with auxin does not prevent NDGA action. NDGA at 50 μM was added at various times (*arrow*) to assays containing 1 μM 2,4-D. ○, No addition; ●, 1 μM 2,4-D alone; ⊗, NDGA added at 0 h; ▲, NDGA added at 1 h; □ NDGA added at 2 h . Inhibition by NDGA is apparent about 1 h after addition

After treatments with 50 μM NDGA for 0.5 h, 1 h, or 2 h, all of the growth curves were shifted to the left in parallel to the control with 2,4-D added alone at 0 h. However, treatment with NDGA for 1 h induced a slightly greater shift and treatment with NDGA for 0.5 h a slightly smaller shift than that induced by adding 2,4-D alone after 1 h. This indicates that recovery from 50 μM NDGA was achieved in less than 0.5 h. Recovery appeared to be complete. When auxin was added first and the inhibitor afterwards, growth inhibition was not detectable until after more than 1 h (Fig. 2B). Hence, neither a very low auxin content of the tissue, as might be possible after cutting and preincubation of control segments, nor the high auxin level applied at the start of an experiment, is a precondition for the inhibitory effect (Fig. 1A,B).

With aristolochic acid, a half-maximal inhibition of elongation growth induced by 1 μM auxin was obtained at 25–50 μM (Fig. 3A,B). In the controls with no added auxin, half-maximal inhibition was obtained at higher concentrations at 50–75 μM (Fig. 3A,B) and 20 μM aristolochic acid had little effect (Fig. 3C). At the higher 2,4-D concentration of 10 μM the inhibitor proved somewhat less effective (Fig. 3C). Full reversibility was obtained and recovery achieved after less than 0.5 h (Fig. 4A). When the inhibitor was added at 20 μM after the addition of auxin the inhibititon was not detected until after more than 1 h (Fig. 4B).

Growth stimulated by IAA and 2,4-D was strongly affected by ETYA (Fig. 5A,B). At 1 μM auxin, half-maximal inhibition was obtained at 20 μM ETYA and 1 μM 2,4-D or IAA Fig. 5A–C). Nearly complete inhibition of growth induced by 1 μM auxin was already obtained by 40 μM ETYA (Fig. 5A, B). At 40–60 μM, ETYA inhibited control growth by about 50% (Fig. 5A, B) whereas 20 μm ETYA had little effect on control growth without hormone (Fig. 5C). Addition of 20 μM ETYA to growth tests with 10 μM, 1 μM, or 0.1 μM 2,4-D caused much stronger inhibition of the 2,4-D-induced growth at the lower 2,4-D concentrations (Fig. 5C,D). As compared to a 1-h-delayed addition of auxin, a 1-h treatment with 20 μM ETYA needed a recovery time of about 1 h (Fig. 6A) so that it was reversible. When 20 μM ETYA was added later to auxin-treated growth assays the onset of inhibition started after more than 1 h (Fig. 6B).

The PLA_2 inhibitor PB_x, a polymeric prostaglandin B derivative (Rosenthal et al. 1992), also inhibited auxin-induced growth (Fig. 7A). Inhibition by PB_x was reversible (not shown). When the molar concentration of PB_x, about 10 μM, is taken into consideration it was more effective than the inhibitor OEPC, a phospholipid analogue (Fig. 7B).

Fusicoccin, a fungal toxin, can partially mimick auxin in that it also induces growth in etiolated stem segments and coleoptile segments (Marrè et al. 1973). For the

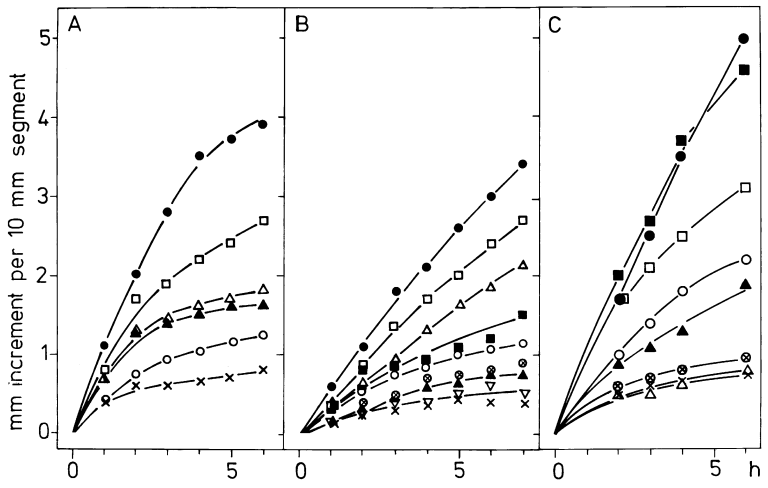

Fig. 3A–C. Influence of different concentrations of aristolochic acid on auxin-induced extension growth of zucchini hypocotyls. **A** IAA at 1 μM in combination with various aristolochic acid concentrations. ○, No addition; ×, 50 μM aristolochic acid alone; ●, 1 μM IAA alone; □, 1 μM IAA + 25 μM aristolochic acid; Δ, 1 μM IAA + 50 μM aristolochic acid; ▲, 1 μM IAA + 75 μM aristolochic acid. **B** 2,4-D at 1 μM in combination with various aristolochic acid concentrations. ○, No addition; ⊗, 50 μM aristolochic acid alone; ×, 100 μM aristolochic acid alone; ●, 1 μM 2,4-D alone; □, 1 μM 2,4-D + 10 μM aristolochic acid; Δ, 1 μM 2,4-D + 25 μM aristolochic acid; ■, 1 μM 2,4-D + 50 μM aristolochic acid; ▲, 1 μM 2,4-D + 75 μM aristolochic acid; ∇, 1 μM 2,4-D + 100 μM aristolochic acid. **C** Different 2,4-D concentrations in combination with 20 μM aristolochic acid. ⊗, No addition; ×, 20 μM aristolochic acid; ■, 10 μM 2,4-D alone; □,10 μM 2,4-D + 20 μM aristolochic acid; ●, 1 μM 2,4-D alone; ○, 1 μM 2,4-D + 20 μM aristolochic acid; ▲, 0.1 μM 2,4-D alone; Δ, 0.1 μM 2,4-D + 20 μM aristolochic acid

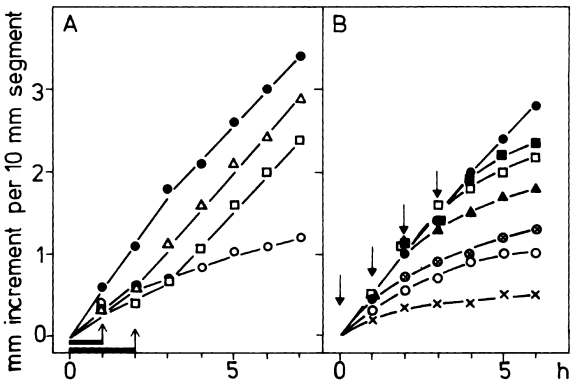

Fig. 4A,B. Reversibility of the inhibitory effect of aristolochic acid on auxin-induced growth of zucchini hypocotyls. **A** 2,4-D at 1 μM was added alone (●), or no addition was made (○), or 1 μM 2,4-D in combination with 50 μM aristolochic acid was added at 0 h. After the times indicated (*bar and arrow*), 2,4-D + aristolochic acid was replaced by 2,4-D alones Δ, after 1 h; □, after 2 h. Growth recovered at slightly more than 1 h after aristolochic acid withdrawal. **B** Pretreatment by auxin does not prevent aristolochic acid action. Aristolochic acid at 50 μM was added at various times (*arrows*) to assays containing 1 μM 2,4-D. ○, No addition; × 50 μM, aristolochic acid alone; ●, 1 μM 2,4-D alone; ⊗ aristolochic acid added at 0 h; ▲, aristolochic acid added at 1 h; □, aristolochic acid added at 2 h; ■, aristolochic acid added at 3 h. Inhibition by aristolochic acid is apparent about 1 h after addition

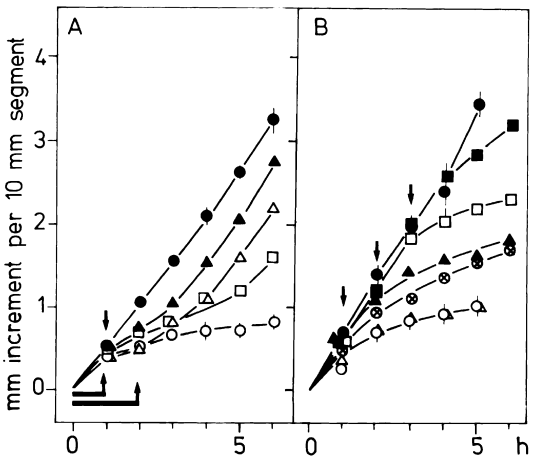

Fig. 6A,B. Reversibility of the inhibitory effect of ETYA on auxin-induced growth of zucchini hypocotyls. **A** 2,4-D at 1 μM was added alone (●, duplicate assays) or in combination with 20 μM ETYA at 0 h. After the times indicated (*bar and arrow*) 2,4-D + ETYA was replaced by 2,4-D alone: Δ, after 1 h; □, after 2 h. For comparison, 2,4-D alone was added 1 h after the start of incubation (*downward pointing arrow*, ▲), or no addition was made (○, duplicate assays). Growth recovered at slightly more than 2 h after ETYA withdrawal. **B** Pretreatment with auxin does not prevent ETYA action. ETYA at 20 μM was added at various times (*arrow*) to assays containing 1 μM 2,4-D. ○, No addition (duplicate assays); ●, 1 μM 2,4-D alone (duplicate assays); Δ, 20 μM ETYA alone; ⊗, ETYA added at 0 h; ▲, ETYA added at 1 h; □, ETYA added at 2 h; ■, ETYA added at 3 h. Inhibition by ETYA is apparent about 1 h after addition

three inhibitors, NDGA, aristolochic acid, and ETYA, we made a direct comparison of their effects on 2,4-D-induced and fusicoccin-induced growth. We chose 10 μM 2,4-D and 0.5–1 μM fusicoccin, in experiments with the same batch of plants, because these concentrations induce similar growth effects. Fusicoccin-induced growth was quite resistant to NDGA and ETYA at concentrations at which auxin-induced growth was strongly affected whereas aristolochic acid inhibited 2,4-D- and fusicoccin-induced growth to a somewhat similar extent (Fig. 8). At 10 μM fusicoccin, inhibitors were almost ineffective (data not shown).

The three inhibitors NDGA, aristolochic acid, and ETYA were chosen for further study of the signal specificity of their effects. In contrast to auxin, where the use of etiolated and cut tissue segments is a necessity, gibberellic acid (GA_3) is a growth-inducing signal in certain plants grown in the light. Garden pea races are often gibberellin-deficient so that they are suitable for such studies. Treatment of imbibed pea seeds with GA_3 resulted in a dramatic increase in the length of intact pea stems grown in the light (Fig. 9A). This GA_3-induced growth could not be suppressed by NDGA or ETYA; however, a high concentration of aristolochic acid (100 μM) damaged not only the GA_3 response but also the growth response of untreated pea seedlings (Fig. 9B–D). Nevertheless, the response to GA_3 as measured by a

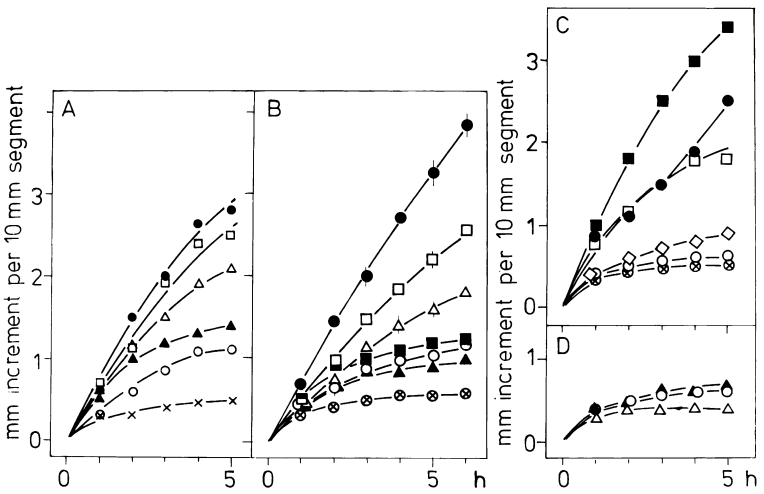

Fig. 5A–C. Influence of different concentrations of ETYA on auxin-induced extension growth of zucchini hypocotyls. **A** IAA at 1 μM in combination with various ETYA concentrations. ○, No addition; ×, 40 μM ETYA alone; ●, 1 μM IAA alone; □, 1μM IAA + 5 μM ETYA; Δ, 1 μM IAA + 20 μM ETYA; ▲, 1 μM IAA + 40 μM ETYA. **B** 2,4-D at 1 μM in combination with various ETYA concentrations. ○, No addition (duplicate assays); ⊗, 60 μM ETYA alone; ●, 1 μM 2,4-D alone (duplicate assays); □, 1 μM 2,4-D + 10 μM ETYA (duplicate assays); Δ, 1 μM 2,4-D + 20 μM ETYA (duplicate assays); ■, 1 μM 2,4-D + 40 μM ETYA; ▲, 1 μM 2,4-D + 60 μM ETYA. In assays done in duplicate, the experimental error is indicated by a bar where it exceeds the size of the symbols. **C, D** Different 2,4-D concentrations in combination with 20 μM ETYA. ○, No addition; ■, 10 μM 2,4-D alone; □, 10 μM 2,4-D + 20 μM ETYA; ●, 1 μM 2,4-D alone; ◇, 1 μM 2,4-D + 20 μM ETYA; ▲, 0.1 μM 2,4-D alone; Δ, 0.1 μM 2,4-D + 20 μM ETYA; ⊗, 20 μM ETYA alone

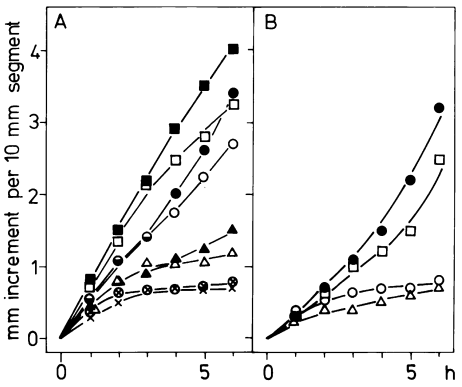

Fig. 7A,B. Influence of PB_x (polymeric prostaglandin B, x = 2–3) and of OEPC (oleyloxyethyl phosphocholine) on auxin-induced extension growth of zucchini hypocotyls. **A** 2,4-D at 0.1 μM, 1 μM and 10 μM in combination with 10 μg · mL^{-1} PB_x. ⊗, No addition; ×, PB_x alone; ■, 10 μM 2,4-D alone; □ 10 μM 2,4-D + PB_x; ●, 1 μM 2,4-D alone; ○, 1 μM 2,4-D + PB_x; ▲, 0.1 μM 2,4-D alone; Δ, 0.1 μM 2,4-D + PB_x. **B** 2,4-D at 1 μM in combination with 100 μM OEPC. ○, No addition; Δ, OEPC alone; ●, 1 μM 2,4-D alone; □, 1 μM 2,4-D + OEPC

comparison of the controls with the hormone treatment, was in no instance completely abolished by aristolochic acid (Fig. 9C).

The radish cotyledon expansion test, in which cotyledons are excised and treated with exogenous cytokinins, e.g kinetin, is a cytokinin-specific biotest. The cotyledons are not sensitive to added auxins. In the same plant, the hypocotyl is sensitive to addition of auxin but not to cytokinins (Letham 1971). When excised radish cotyledons were treated with 2 μM kinetin their fresh weight increased by about 65% in comparison to buffer-treated controls (Fig. 10). The inhibitors NDGA at 100 μM, and ETYA at 40 μM did not influence the kinetin response, and 80 μM ETYA was rather toxic, as was especially apparent from the only partial greening and the lack of cotyledon expansion. Aristolochic acid showed this toxicity at 50 and 100 μM in this test.

Discussion

Remarkably, five out of five substances known to inhibit animal PLA_2 enzymes inhibited auxin-induced extension growth in etiolated zucchini hypocotyl segments. Knowledge about the different animal PLAs, their functions and their inhibitors, is rapidly accumulating due to the great potential impact of arachidonate metabolism in a wide array of diseases and in cancer (Dennis 1994). It seems clear that the cytosolic Ca^{2+}-independent PLA_2 (group IV) functions solely in signal transduction by generating the arachidonic acid which is modified by lipoxygenase and cyclooxygenase to leukotrienes and prostaglandins (Samuelson et al. 1987). At least one additional pathway for arachidonic acid release is provided by the so-called group II small Ca^{2+}-dependent, secreted PLA_2 enzymes during inflammatory reactions whereas other small Ca^{2+}-dependent, secreted

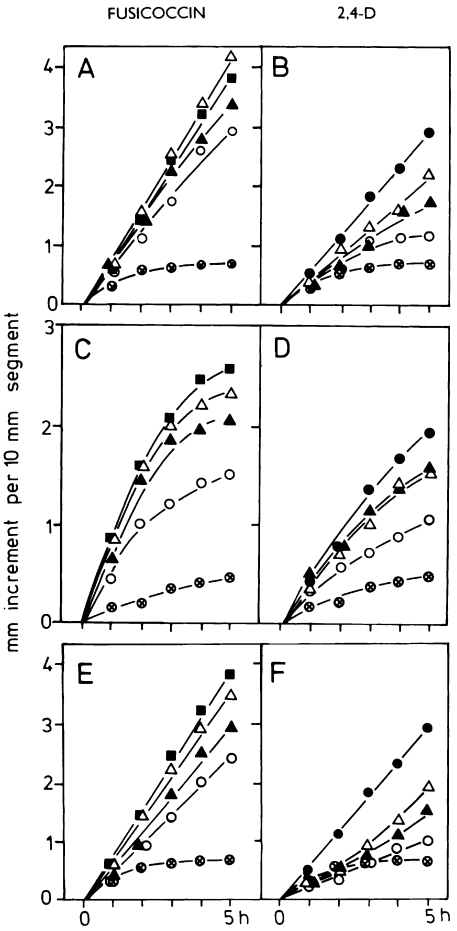

Fig. 8A–F. Influence of different concentrations of NDGA (**A, B**), aristolochic acid (**C, D**) and ETYA (**E, F**) on fusicoccin-induced (*left panels*) and 2,4-D-induced growth (*right panels*) of zucchini hypocotyls. **A** ⊗, No addition; ■, 0.5 μM fusicoccin alone; Δ, 0.5 μM fusicoccin and 25 μM NDGA; ▲, 0.5 μM fusicoccin and 50 μM NDGA; ○, 0.5 μM fusicoccin and 100 μM NDGA. **B** ⊗, No addition; ●, 10 μM 2,4-D alone; Δ, 10 μM 2,4-D and 25 μM NDGA; ▲, 10 μM 2,4-D and 50 μM NDGA; ○, 10 μM 2,4-D and 100 μM NDGA. **C** ⊗, No addition; ■, 1 μM fusicoccin alone; Δ, 1 μM fusicoccin and 10 μM aristolochic acid; Δ, 1 μM fusicoccin and 10 μM aristolochic acid; ▲, 1 μM fusicoccin and 25 μM aristolochic acid; ⊗, 1 μM fusicoccin and 50 μM aristolochic acid. **D** ⊗, No addition; ●, 10 μM 2,4-D alone; Δ, 10 μM 2,4-D and 10 μM aristolochic acid; ▲, 10 μM 2.4-D and 25 μM aristolochic acid; ○, 10 μM 2,4-D and 50 μM aristolochic acid. **E** ⊗, No addition; ■, 0.5 μM fusicoccin alone; Δ, 0.5 μM fusicoccin and 20 μM ETYA; ▲, 0.5 μM fusicoccin and 40 μM ETYA; ○, 0.5 μM fusicoccin and 80 μM ETYA. **F** ⊗, No addition; ●, 10 μM 2,4-D alone; Δ, 10 μM 2,4-D and 20 μM ETYA; ▲, 10 μM 2,4-D and 40 μM ETYA; ○, 10 μM 2,4-D and 80 μM ETYA

PLA_2 enzymes (group I) seem to have a role in digestion or as venoms only.

In higher plants, a pathway very similar to the generation of prostaglandins, leading from linolenic acid to jasmonate, is present but arachidonate, the precursor fatty acid to prostaglandins in animal cells, is absent (Anderson 1989). The sparse knowledge about plant PLAs does not yet allow conclusions to be drawn about the nature of the enzyme or enzymes that may provide linolenic acid and lysophospholipids as the potentially biologically active lipid metabolites in plants (Scherer

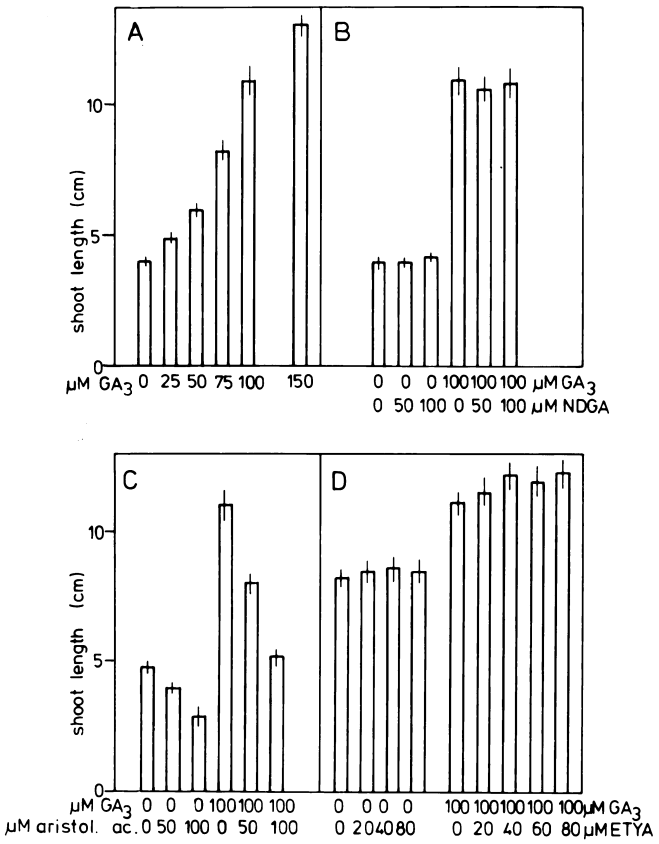

Fig. 9A–D. Influence of NDGA, aristolochic acid, and ETYA on GA_3-induced shoot growth in light-grown peas. **A** Influence of increasing GA_3 concentrations alone. **B** Influence of NDGA on shoot growth in control peas without GA_3 (*first three columns*) or in peas treated with 100 μM GA_3 (*second three columns*). **C** Influence of aristolochic acid on shoot growth in control peas without GA_3 (*first three columns*) or in peas treated with 100 μM GA_3 (*second three columns*). **D** Influence of ETYA on shoot growth in control peas without GA_3 (*first four columns*) or in peas treated with 100 μM GA_3 (*second four columns*). Data are means ± SE, $n = 35$

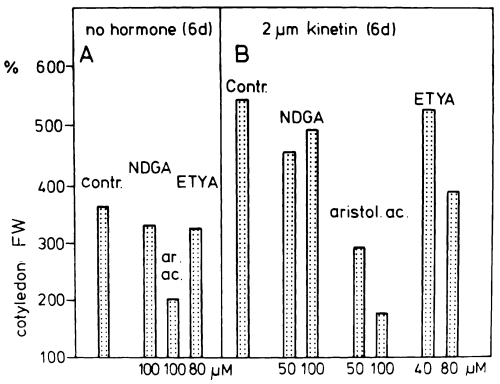

Fig. 10A,B. Influence of NDGA, aristolochic acid, and ETYA on kinetin-induced expansion growth of excised radish cotyledons. Growth is expressed as increase in fresh weight, where 100% is the weight of a batch of ten cotyledons at the time of cutting (50 ± 5 mg). **A** Treatments for 6 d in buffer. **B** Treatments for 6 d in buffer plus 2 μM kinetin

1996). The group of small 14-kDa PLA_2 enzymes could be present in plants (Kim et al. 1994) as well as digestive enzymes in the vacuole (Racusen 1984) but, most likely, other different phospholipases of the A-type are to be expected in plants (Gaillard 1980; Huang 1987). Signal transduction could be exerted by an enzyme homologous to animal enzyme(s) or by an as yet unknown plant PLA (Creelman and Mullet 1995).

The three inhibitors used mostly in this study, NDGA, aristolochic acid, and ETYA, are all inhibitors of the animal cytosolic PLA_2, which functions in the release of arachidonic acid, but some of these inhibitors may have additional effects on other enzymes. Aristolochic acid has been used most often in animal studies and inhibits not only 14-kDa PLA_2 enzymes (group I and II) but also the release of arachidonic acid with an IC_{50} of 40 μM in animal cells, likely due to inhibition of cytosolic phospholipase (Group IV) (Vishnawath et al. 1988; Rosenthal et al. 1992; Sa and Fox 1994). Nordihydroguajaretic acid strongly inhibits the release of arachidonic acid at 40 μM but also has additional effects on lipoxygenase (Gerrard 1985; Ondrey et al. 1989; Hannigan and Williams 1991; Ponzoni et al. 1992). Not only does ETYA inhibit arachidonic release with an IC_{50} of about 17 μM but it also inhibits this process almost completely at 32 μM (Ondrey et al. 1989); moreover, it is an inhibitor of subsequent oxygenation (Hannigan and Williams 1991). Inhibition of lipoxygenase by ETYA is irreversible (Kühn et al. 1984) whereas the physiological mode of action on growth was reversible in this study. For PB_x, inhibitition of release of arachidonic acid has been demonstrated (Rosenthal et al. 1992). Oleyloxyethyl phosphocholine inhibits pancreatic (group I) secreted PLA_2 (Magolda and Galbraith 1989) but may also inhibit other PLA_2 activities since all PLA_2 inhibitors seem to have a broad specificity towards different animal phospholipases of the A_2-type. In summary, the chemical structures of the inhibitors used in this study are so diverse yet their specificity is sufficiently narrow that application of these compounds at low concentrations in plants should inhibit only those metabolic pathways concerning the liberation of fatty acids and, perhaps, their subsequent metabolism by lipoxygenase and cyclooxygenase. Hence, growth inhibition by these compounds indicates that the liberation of fatty acids is a key event in the growth response of cut etiolated stem segments to auxin and strengthens our suggestion that receptor-mediated activation of a PLA activity releases fatty acids and lysolipids or derivatives thereof as potential second messengers in plants (Scherer and André 1989, 1993; André and Scherer 1991). A rather similar conclusion has been reached about auxin-induced acidification and growth in maize coleoptiles which is partially mimicked by fatty acids and lysophosphatidylcholine (Yi et al. 1996) and about elicitor-induced PLA which is involved in triggering the oxidative burst (Chandra et al. 1996).

The extent of the similarity and dissimilarity between the effects of fusicoccin and auxin on elongation growth and acidification is still a matter of debate (Schopfer 1993). Yi et al. (1996) observed a qualitative difference

between the effects of the PLA inhibitors aristolochic acid and monoalide on acidification by auxin vs. fusicoccin, both being ineffective in the case of fusicoccin. In growth tests with auxin, these inhibitors were also effective but were not tested on fusicoccin-induced growth by these authors. In our system, aristolochic acid affected fusicoccin-induced growth and 2,4-D-induced growth to a rather similar extent but NDGA and ETYA were fairly good auxin-specific inhibitors. Phospholipase A activities may be necessary not only in signal transduction reactions but also in phospholipid metabolism which, in turn, could be vital for secretion or other processes. Secretion is necessary in auxin-induced elongation growth (Hager et al. 1989, 1991; Schindler et al. 1994) but has not been investigated with fusicoccin. Moreover, the inhibitor specificities are certainly not absolute for signal transduction by PLA so that overlapping functions or processes are likely triggered in rapid cell expansion by auxin and fusicoccin. A case for overlapping functions or reactions can be made by the recent findings that the fusicoccin receptor is a 14-3-3 protein (Korthout and De Boer 1994; Oecking et al. 1994) and that 14-3-3 proteins bind to phosphorylated serine residues surrounded by a certain binding motif in proteins with diverse functions in signal transduction (Muslin et al. 1996). Although as yet speculative for plants, this would indicate that binding of the fusicoccin receptor to one or several phosphorylated proteins could interfere with existing signal transduction pathways, the auxin pathway or pathways being be the most likely target (Korthout and De Boer 1994).

Extension growth is a process which is influenced positively by several hormones, auxin, gibberellic acid and cytokinin, negatively by others (abscisic acid, jasmonic acid) and negatively by red and blue light. Clearly, extension growth is part of morphogenic processes and governed by endogenous or exogenous signals which "allocate" the ability to expand in a differentiation-dependent (e.g. light-grown vs. dark-grown) or a tissue-dependent manner so that signal-specific growth situations, e.g. those due either to auxin, or gibberellin, or cytokinin, are possible. Therefore, the effects of the three inhibitors NDGA, ETYA, and aristolochic acid were compared in two further typical biotests, one for gibberellin-induced stem extension growth in light-grown intact peas (Kende and Lang 1964), and one for cytokinins, the cotyledon expansion test with excised cotyledons (Letham 1971). In these two additional biotests the three inhibitors were either not effective or were much less effective, so that we hypothesize that signal transduction by GA_3 or by kinetin is different from auxin signal transduction, i.e. that a PLA pathway is, functionally at least, much less important for these two hormones.

Receptors and the receptor-mediated signal transduction steps, e.g. the generation of intracellular second messengers or activation of protein kinases or phosphatases, are the basis for specificity in signal transduction. Complex physiological parameters such as growth are influenced by several signals and these are most likely integrated somehow at the level of the intermediate reactions in signal transduction. Therefore, we suspect that the key signal intermediates for auxin, on the one hand, and for GA_3 and cytokinin, on the other hand, are different, and may be more similar for auxin and fusicoccin.

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