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Sensory Adaptation in Polar Auxin Transport System to Naphtylphthalamic Acid in Corn Coleoptile Segments

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옥수수 (*Zea mays* L.) 자엽초 절편에서 Naphtylphthalamic Acid에 대한 오옥신 이동계의 감지적응

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ABSTRACT

Partial recovery in auxin transport capacity from inhibition by *N*-1-naphthylphthalamic acid (NPA) was observed when corn coleoptile segments were subjected to a prolonged NPA treatment. Kinetic data indicated that the recovery time is a function of the concentration of NPA applied. Desensitization to NPA was also seen in tissue slices where NPA increased net uptake of auxin, indicating that the apparant adaptation in the auxin transport system did not result possibly from auxin accumulated during transport inhibition. Studies on *in vitro* binding of NPA to membrane vesicles isolated from the coleoptile indicated that preincubation of the tissue with NPA resulted in the reduced binding activity. Scatchard analysis of the data indicated that this was due to decreases in the number of NPA binding sites. The possibility of causal relationship of modified NPA receptors to the sensory adaptation in auxin transport observed in coleoptile segments will be discussed.

INTRODUCTION

The phenomenon of sensory adaptation is well known in biological systems. Cells exposed to a specific stimulus for a prolonged period often lose or diminish the ability to respond to the stimulus. This sensory adaptation or desensitization plays an important role in bacterial chemotaxis or hormonal responses in certain animal systems. Biochemical or molecular basis for sensory adaptation such as receptor down regulation (Siebly *et al.*, 1985) or covalent modifications (Springer *et al.*, 1979) is also well characterized in these systems.

In plants, although molecular mechanisms are yet to be elucidated, physiological studies on adaptation to plant hormones in gravitropic (Salisbury *et al.*, 1988; Evans, 1991) or growth (Gougler and Evans, 1981) responses are well documented. Changes in the sensitivity of plant tissues to growth substances are thought to play an important regulatory role in plant growth and development (Trewavas, 1981).

Auxin-response systems partially sensitized (Vesper and Evans, 1979) and desensitized, i.e., adapted (Gougler and Evans, 1981) to changes in auxin concentration have been described. Hertel (1983) proposed a molecular model for auxin action where a reversible, covalent modification of auxin receptors is involved in interconversion of sensitive and adaptive states. The model depicts computer-simulated gating cycles, which also explains carrier-mediated auxin transport across the membrane. However, experimental evidence on adaptation in auxin transport system is limited to one briefly documented case (Hertel, 1985).

In this paper, we present further physiological evidence on adaptation of auxin transport system to NPA, the specific inhibitor of polar auxin transport (Thomson and Leopold, 1974), using two different experimental systems.

MATERIALS AND METHODS

Plant material. Presoaked corn (*Zea mays* L.) seeds were planted on wet paper towels in plastic trays and placed vertically in the dark at 28℃ with saturated humidity. When seedlings were 4-5 day old, subapical coleoptile segments 3-10 mm in length were cut with a double blade cutter and primary leaves removed. These segments were used in all experiments. In the case where sunflower (*Helianthus annuus* L.) hypocotyls were used, subapical hypocotyl segments 10 mm in length were cut from 6-7 day old dark grown seedlings.

Radiochemicals. (5-^{3}H)-IAA (28 Ci/mmole) and (2, 3, 4, 5-^{3}H)-NPA (55 Ci/mmole) were purchased from CEA (Gif-sur-Yvette, France).

Auxin transport test. Auxin transport tests were carried out at 28℃ under dim green light. Agar blocks (1.5%, 3 mm×3 mm×1 mm, with 50 mM sodium phosphate buffer, pH 6.8) containing 3.8×10^{-8} M ^{3}H-IAA were used as donor blocks. Receiver blocks contained plain buffered agar or test chemicals where indicated. Individual segments were placed vertically, basal end down, between donor (apical end) and receiver (basal end) blocks. At the end of a transport period, the radioactivity in the receiver block was counted with liquid scintillation spectrometry.

Net uptake of ^{3}H-IAA. Net uptake of auxin was measured as described previously by Edwards and Goldsmith (1980). Subapical corn coleoptile tissues were cut into 1 mm slices with a multibladed cutter. Twenty slices were incubated in 1 m*l* sodium phosphate buffer (10 mM, pH 5) containing 8.9×10^{-9} M ^{3}H-IAA and test chemicals in a 20 m*l* vial. At the end of an uptake period (ca. 30 min) the medium was rapidly removed and slices were washed twice with cold buffer under reduced pressure. Ten slices were transferred to a scintillation vial and the radioactivity was counted.

NPA binding tests. All procedures were carried out at 4℃ according to the method described by Michalke and Schmieder (1979). Coleoptiles were chopped and ground with the extraction medium (4 m*l*/g.f.w.). The extraction medium contained 5 mM HEPES, 30 mM $CaCl_2$, 10 mM $MgCl_2$, 0.5 M NaCl, 50 mM EDTA, pH 5.3 and 14 mM β-mercaptoethanol. Crude homogenates were squeezed through a nylon cloth and centrifuged for 20 min at 3000 rpm (1,000 g). To the supernatant, 10% PEG was added and centrifuged again (3000 rpm×30 min). Membrane materials were partitioned with PEG at the low centrifugal force. The membranes were resuspended in the assay medium (5 mM HEPES, 30 mM $CaCl_2$, 10 mM $MgCl_2$, 0.5 M NaCl, pH 5.3: 1 m*l*/g.f.w.). ^{3}H-NPA (5×10^{-10} M) was added to the resuspended particles (200 μ*l*) with or without various concentrations of cold NPA. After a 30 min incubation with constant stirring, 10 m*l* of the cold assay medium was poured to the binding mixture and immidiately filtered through a membrane filter (0.45 μm pore size, 47 mm diameter, Satorious membrane filter, Gottingen, FRG) under reduced pressure. The filter was washed rapidly with another 10 m*l* of the wash medium, and air dried. The radioactivity on the filter was counted.

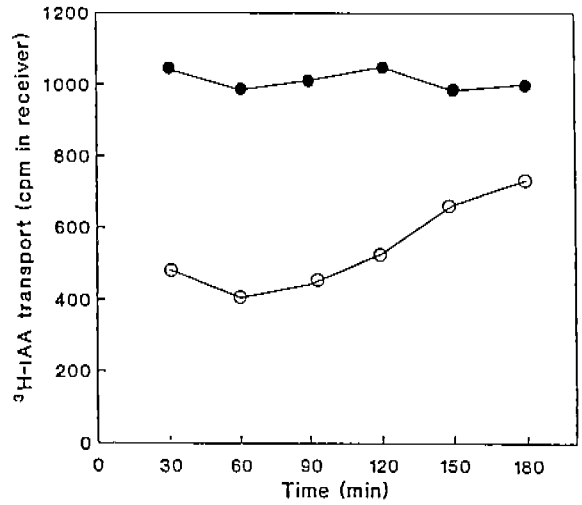

Fig. 1. Transport of ^{3}H-IAA and its inhibition by NPA. Subapical coleoptile segments (3 mm) were preloaded with ^{3}H-IAA (0.25 μCi/m*l*) for 1 h. The segments were then transferred to fresh receivers with (○) or without (●) 0.3 μM NPA every 30 min. Data represent average values of three duplicate experiments.

RESULTS AND DISCUSSION

Adaptation to NPA in transport inhibition. Polar auxin transport is strongly and rapidly inhibited by the phytotropin NPA. In our present work, the inhibitory effect of NPA was measured at various times during a prolonged period of treatment with the phytotropin. A low concentration of NPA (0.3 μM) was used for partial inhibition of auxin transport. Subapical coleoptile segments were preloaded with ^{3}H-IAA (donor) for 1 h before transport test in the presence or absence of NPA (receiver). The segments were then transferred to fresh receivers (with or without NPA) every 30 min. During the

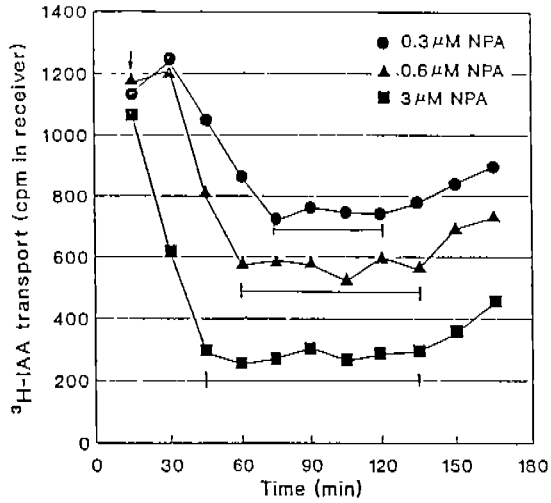

Fig. 2. Time course for the transport inhibition by NPA. Subapical corn coleoptile segments (3 mm) were preloaded with ^{3}H-IAA (0.25 μCi/m*l*) for 30 min. The segments were then transferred to fresh receivers containing indicated concentrations of NPA every 15 min. Data represent average values of two duplicate experiments. The arrow indicates the time of NPA application. The horizontal bars indicate the time required for recovery of the transport capacity from the inhibition by NPA.

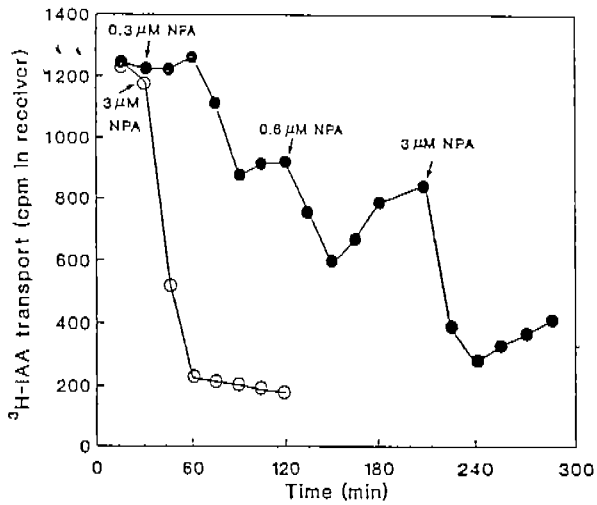

Fig. 3. Time course for the transport inhibition by NPA in coleoptile segments previously exposed to NPA. Subapical segments (3 mm) were preloaded with ^{3}H-IAA (0.25 μCi/m*l*) for 30 min. The segments were then transferred to either fresh receiver with 3 μM NPA (○) or receivers with increasing concentrations of NPA from 0.3 μM to 3 μM NPA (●). Data represent average values of two duplicate experiments. The arrows indicate the time of NPA application.

first 30 min period of NPA treatment, about 50% inhibition of auxin transport was brought about by NPA at this concentration. However, the transport capacity in the presence of NPA was recovered as the NPA-treatment time increased (Fig. 1), suggesting that the tissue might undergo desensitization to NPA. This "apparent adaptation" to NPA seems to depend on NPA concentrations, i.e., the time required to recover the transport capacity from the inhibitory effect of NPA was prolonged with increasing concentration of NPA applied (Fig. 2). When one compares the time courses of transport inhibition by NPA in Fig. 2, both the time required for full activity of NPA and that for recovery of the transport capacity from the inhibition by NPA show a concentration dependency. NPA has been known to inhibit auxin transport very quickly, within minutes (Thomson *et al.*, 1973). This is the case when the NPA concentration was sufficiently high. However, from our results in Fig. 2, it is clear that a low concentration of NPA rather stimulates auxin transport at first. After this initial phase, NPA starts to inhibit transport, and about 1 h was needed for full NPA activity at 0.3 μM. It was also observed that very low concentrations of NPA ($10^{-9}-10^{-8}$ M) had a stimulatory effect on auxin transport (data not shown), indicating that NPA effect on auxin transport could be reversed depending on its concentration. This may have relevance to recent findings on phytotropin binding sites having two, functionally different binding sites with high and low affinities to NPA, respectively (Michalke *et al.*, 1989).

Fig. 3 illustrates that auxin transport was inhibited step-by-step with gradually increasing concentrations of NPA from 0.3 to 3 μM. Compared with the control where auxin transport was initially inhibited by 3 μM NPA (Fig. 3, left), the transport capacity was more rapidly recovered from the inhibition in the coleoptile segments which were gradually "pre-exposed" to NPA. This shortening of the recovery time suggests that the tissue could "remember" and be adapted to the pre-exposure of NPA. However, the degree of transport inhibition by 3 μM NPA was almost same in both cases and the recovery was only partial, raising the question whether or not the sensitivity of the tissue to NPA was really changed. Because NPA is known to stimulate net accumulation of auxin in tissue segments (Sussmann and Goldsmith, 1981), one might argue that this "apparant adaptation" could be a reslt from a pile-up of auxin in the tissue by NPA. In such

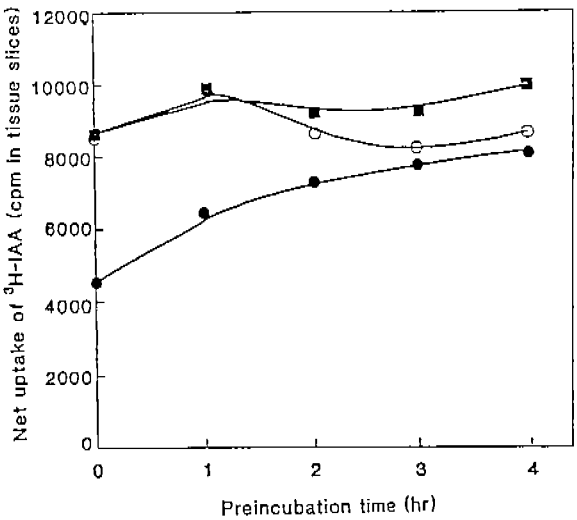

Fig. 4. Effect of NPA pretreatment on subsequent NPA action on ^{3}H-IAA uptake by coleoptile slices. Subapical segments (1 cm) were preincubated for indicated time periods with or without 10 μM NPA. At the end of these periods, 1 mm slices were cut from these segments and net uptake of ^{3}H-IAA (0.25 μCi/ml, pH 5) was measured. Cpm values were obtained from tissues preincubated in the absence (closed symbols) or presence (open symbols) of 10 μM NPA. NPA was either present (closed squares and open circles) or absent (closed circles) in the uptake medium. Data represent average values of four duplicate experiments.

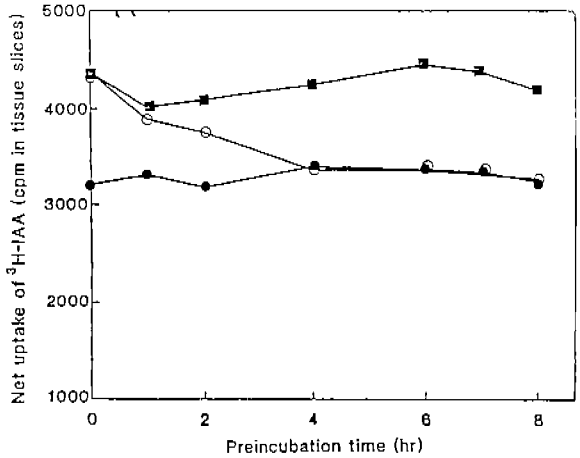

Fig. 5. Effect of NPA pretreatment on subsequent NPA action on ^{3}H-IAA uptake by sunflower hypocotyl slices. Subapical segments (1 cm) were preincubated for indicated time periods with or without 10 μM NPA. At the end of these periods, 1 mm slices were cut from these segments and net uptake of ^{3}H-IAA (0.25 μCi/ml, pH 5) was measured. Cpm values were obtained from tissues preincubated in the absence (closed symbols) or presence (open symbols) of 10 μM NPA. NPA was either present (close squares and open circles) or absent (closed circles) in the uptake medium. Data represent average values of two duplicate experiments.

a case, the recovery of auxin transport from the inhibition by NPA must be a function of transportable auxin accumulated in the tissues and the inhibitory action of NPA. However, the relative importance of increased auxin content by NPA in the transportable auxin pool remains unknown and there is a report indicating that the increased auxin content in pea stem tissue by NPA is mainly nontransportable (Lee, 1981). Adaptation in the auxin transport system to the soft inhibitor 3,4,5-TIBA was also reported not due to a pile-up of auxin (Hertel, 1985), suggesting that the increased auxin content by transport inhibitors has a neglegible effect on the polar auxin transport system in corn coleoptile segments.

Adaptation in net uptake of auxin to NPA. In order to minimize the problem of possible interference of transportable auxin in the adaptation of auxin transport system to NPA, we used an experimental system in which net uptake of auxin by thin (1 mm) coleoptile sections was measured. This system is known to be basically the same as the conventional polar auxin transport system using longer segments with agar blocks. Net uptake of auxin by tissue sections is based upon pH dependent transmembrane transport consisting of influx and efflux of auxin via saturable carriers (Edwards and Goldsmith, 1980). NPA is known to stimulate net uptake of auxin by specifically inhibiting auxin efflux carriers (Sussmann and Goldsmith, 1981).

Subapical coleoptile segments were preincubated for various periods with or without NPA and transferred to fresh medium to measure NPA-stimulation of auxin net uptake. Results of the experiment are shown in Fig. 4. For maximal activity, 10 μM NPA was used. Compared with the untreated control, the stimulatory effect of NPA was decreased as the time of NPA-pretreatment increased. This is not a result of a decreased electrochemical gradient, the driving force of auxin uptake, due to prolonged NPA pretreatment because preincubation with other weak acids did not influence on the subsequent NPA effect (data not shown). The auxin uptake by corn coleoptile sections seems to be a complicated system because net uptake represents a result of two opposing processes, namely influx and efflux of auxin at the cellular level,

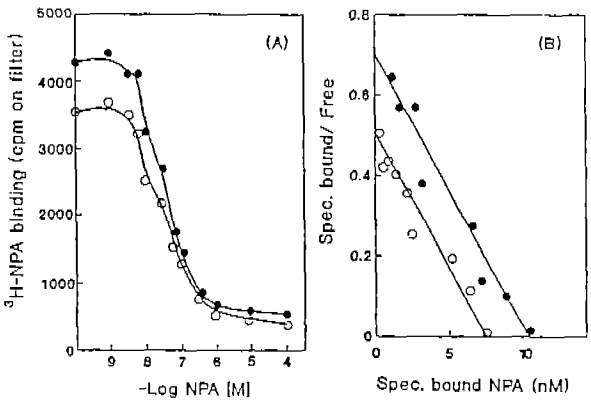

Fig. 6. Dissociation curve (A) and Schatchard plot (B) of ^{3}H-NPA binding to membranes from coleoptile tissues. Subapical segments (1-2 cm) were preincubated for 4 h in the presence (●) or absence (○) of 10 μM NPA, PEG-supported membrane particles were obtained and washed twice for ^{3}H-NPA binding assay. Data represent average values of two duplicate experiments.

and the activity of auxin efflux carrier is known to rapidly decrease with preincubation time (Yoon, 1991). This could explain the time-dependent increases in auxin uptake and decreases in NPA effect in the untreated (minus NPA) control as shown in Fig. 4. However, when the segments were preincubated with NPA, the decrease in NPA effect was more rapid and greater than the control, indicating that the process of time-dependent changes in auxin transport system and adaptation to NPA could be separated. This is supported by the result shown in Fig. 5, where sunflower hypocotyl segments were used instead of corn coleoptiles. Auxin transport decay in the sunflower hypocotyl segment was known to occur very slowly (de La Funte and Leopold, 1970) compared with corn coleoptiles. As shown in Fig. 5, neither increases in auxin uptake nor decreases in NPA effect were observed in NPA-non-pretreated control within the range of experimental period tested. However, a decrease and eventual dissapearance of NPA effect were clearly seen in the hypocotyl segments as the NPA-pretreatment time increased, indicating that the tissue underwent desensitization to NPA.

The NPA-sensitive increase in auxin uptake is directly correlated with the activity of auxin transport system, i.e. auxin efflux carrier (Hertel *et al.*, 1983). Thus, the results in Figs. 4 and 5 clearly indicate that the auxin transport system exposed to NPA for a prolonged period is adapted to NPA. The exposure time needed to have the system adapted was about 3-4 h (Figs. 4 and 5).

Table 1. Partial recovery of ^{3}H-NPA binding to membrane from NPA-pretreated corn coleoptile tissues by succesive washing and repelleting

No. of washing	^{3}H-NPA specific binding* (cpm)
0	981
1	1949
2	2126
3	2054

*Difference in ^{3}H-NPA binding (cpm) between plus and minus 10 μM unlabelled NPA.
Subapical segments (1-2 cm) were preincubated for 4 hr with or without 10 μM NPA. PEG-supported membrane particles were obtained and washed for ^{3}H-NPA binding. Data represent average values of two duplicate experiments.

Possible mechanism for transport adaptation to NPA.

Although NPA is a synthetic compound, the plasma membrane of plant cells is known to have NPA receptors to which NPA specifically binds with high affinity (Lembi *et al.*, 1971). Other transport inhibitors are also thought to act via the NPA receptor in the plasmmalemma (Thomson *et al.* 1973, Thomson and Leopold 1974). These compounds are called phytotropins (Katekar and Giessler, 1980). The physiological significane of the NPA receptor is evaluated from the recent finding that some naturally occurring compounds act as NPA agonist, indicating that auxin transport may be regulated by these natural substances through NPA receptor (Rubery and Jacobs, 1990).

In order to elucidate the possible mechanism for transport adaptation, *in vitro* NPA binding to the plasma membranes was measured. Schatchard analysis indicates that preincubation of the tissue with NPA for 4 h resulted in a reduced number of the NPA receptor (Fig. 6). The possibility that reduction of NPA receptor was due to possible remnants of cold NPA pretreated was excluded because the membrane particles were washed twice during preparation. Data in Table 1 show only a small recovery of binding activity by washing the membranes with no further recovery thereafter.

The adaptation phenomena in animal or bacterial systems are mainly explanined by changes in the sensory receptor. Receptor down regulation is one kind of such mechanisms. Likewise, altered NPA receptor could explain the adaptation of the auxin transport system to NPA. The idea is supported by the result in Fig. 6. According to Kang (1986), reduction of NPA binding activity in pea stem segments preincubated with 3,4,5-TIBA occurs more rapidly than non-pretreated controls. The reduction of

the NPA receptor might be related with the previously reported transport adaptation to 3,4,5-TIBA, suggesting that the site of adaptation is the receptor itself. Transport inhibitors, as well as endogenous ligands of NPA receptor could change the receptor to undergo sensory adptation for homeostatic regulation of auxin transport in plant tissues. However, the mechanism by which NPA and other transport inhibitors alter the NPA receptor remains to be elucidated.

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적 요

옥수수(*Zea mays* L.) 자엽초 조직 절편의 오옥신 이동능은 NPA에 의해 현저히 억제된다. 그러나 NPA를 처리한 시간이 길어짐에 따라 조직 절편의 오옥신 이동능이 NPA에 의한 억제작용으로부터 점차적으로 회복된다는 결과를 얻었다. 오옥신 이동능이 회복되는데 걸리는 시간은 처리한 NPA 농도에 의존적으로써, NPA 농도가 낮을수록 회복되는 시간이 짧아진다는 것을 알 수 있었다. 이처럼 NPA가 지속적으로 있음으로써, 조직절편의 NPA에 대한 반응이 점차 감소하는 현상은 자엽초 조직 1 mm 박편에서의 오옥신 축적을 측정한 실험 결과에서도 뚜렷하게 나타났다. NPA는 식물조직 밖으로 오옥신의 유출을 특이하게 억제하기 때문에 결과적으로 조직내 오옥신의 축적을 현저히 증가시킨다. 그런데 이러한 NPA의 촉진효과 역시 NPA를 전처리한 시간이 길어질수록 감소하여, 4시간 동안 NPA를 전처리한 조직 절편에서는 NPA의 효과가 완전히 사라짐을 확인할 수 있었다. 이런 결과들은 옥수수 자엽초 조직의 오옥신 극성 이동계가 그 리간드인 NPA를 감지하여 적응할 수 있다는 것을 시사한다. 또한 NPA 결합을 측정한 실험에서 NPA의 전처리에 의한 NPA 수용체의 수가 감소한다는 결과를 얻었는데, 이는 동물이나 미생물에서 잘 알려진 것처럼, 오옥신 이동계의 NPA에 대한 적응현상이 그 수용체의 변화에 기인하였을 가능성을 보여준다.

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