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Primary Action of Indole-3-acetic Acid in Crown Gall Tumors

INCREASE OF SOLUTE UPTAKE

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ABSTRACT

Exogenously added indole-3-acetic acid at a concentration of 100 micromolars stimulates D-glucose uptake (or 3-*O*-methyl-D-glucose uptake) by 25% in crown gall tumors induced on potato tuber tissue by *Agrobacterium tumefaciens* strain C 58. The titration of the endogenous IAA with the auxin antagonist 2-naphthaleneacetic acid at 100 micromolars reduces D-glucose uptake by about 80%. The apparent inhibition constant K_i is 21 micromolars. Other auxin antagonists like 1-naphthoxyacetic acid and 2-(*p*-chlorophenoxy)-2-methylpropionic acid show similar effects. The uptake of the amino acids leucine, methionine, tryptophan, lysine, and aspartic acid is also inhibited by 2-naphthaleneacetic acid to similar degrees. The auxins 1-naphthaleneacetic acid and 2-naphthoxyacetic acid at concentrations between 10 and 100 micromolars inhibit solute uptake only slightly (inhibition less than 20%). The impact of the results on the postulated role of indole-3-acetic acid as a modifier of the electrochemical proton gradient across the plasmalemma in crown gall tumor tissue is discussed.

It is well established that during the logarithmic growth phase of crown gall tissues from various dicotyledonous species their IAA content increases strikingly (5, 20, 25, 26). Recent evidence supports the idea that part of the accelerated hormone synthesis may be regulated by the transferred DNA originating from the T-region of the T_i plasmid of *Agrobacterium tumefaciens* (1). Earlier work suggested that a decreased destruction of IAA in crown gall tissue, presumably due to the presence of phenolic auxin protectors, is responsible for the auxin accumulation (5, 23, 24). There does not appear to be a qualitative change in the biosynthetic pathway of IAA. It proceeds from L-tryptophan via indole-3-pyruvic acid and/or tryptamine (3, 4) to indole-3-acetaldehyde which may either be reduced to indole-3-ethanol or oxidized to IAA (4; T. Rausch, unpublished results).

Little is known about the function of IAA in crown gall tissues. However, it may be assumed that in tumor tissue IAA causes an increased electrochemical proton gradient across the plasmalemma in a similar way as it does in the classical coleoptile system (7) and thus stimulates the symport of organic solutes with protons into the cells (8, 10, 12, 14, 15, 17, 21). An enhanced uptake of organic nutrients certainly is a prerequisite for the establishment of an efficient metabolic sink if the tumor develops on a host plant.

To verify this assumption it is necessary to test the uptake of organic solutes in the presence and in the absence of the endogenous IAA. In the classical coleoptile system, the endogenous IAA is usually lowered by extensive washing of the tissue prior to the addition of exogenous IAA (7). This method has obvious limitations when the tissue investigated actively synthesizes IAA. Therefore, we used a different approach testing the effect of low concentrations of specific auxin antagonists on the uptake of glucose and amino acids. From extensive work on specific auxin binding to both plasmalemma and tonoplast (11, 13, 22) it is known that the auxin antagonists chosen for the present study may displace bound IAA competitively from the binding sites.

It proved to be possible to neutralize the exogenous IAA *in vivo* by increasing stepwise the concentration of the antagonist and, thus, indirectly demonstrate the drastic modulation of hexose and amino acid uptake by IAA via its influence on the electrochemical proton gradient across the plasmalemma.

MATERIALS AND METHODS

Chemicals. All chemicals were of highest purity available. OMG[1], 1-NAA, 2-NAA, 2-NOA, PCIB, IAA (Sigma). 3-*O*-Methyl-D-[U-^{14}C]glucose, 12.06 MBq/mmol; L-[4,5-^{3}H]leucine, 183.30 MBq/mmol (New England Nuclear). L-[U-^{14}C]methionine, 10.45 MBq/mmol; L-[methylene-^{14}C]tryptophan, 2.13 MBq/mmol; D-[U-^{14}C]glucose, 10.85 MBq/mmol (Amersham). L-[U-^{14}C]lysine, 8.94 MBq/mmol (CEA France).

Plant Material. Tumors were initiated on potato tuber slices using *Agrobacterium tumefaciens*, strain C 58, as described elsewhere (2). The tumors were cultivated in the dark at 23°C. After 28 d the tumors had an average diameter of 2 mm. Tumors of uniform size were selected and removed carefully from the underlying tissue with a microspatula. They were collected in ice-cold washing buffer (10 ml/g fresh weight). Composition of buffer: 1 mM KH_2PO_4, 100 μM $CaCl_2$, 50 mM KCl, adjusted to pH 6.0 with 1 N KOH. Immediately after harvest the tumors were filtered through a nylon sieve (pore width 100 μm) and washed twice with the same volume of fresh buffer to remove cellular debris. Finally, the tissue was degassed in fresh buffer and then collected on a nylon sieve. The tumors were kept on ice until incubation. Harvesting and weighing was completed within 1 h.

Test System. 100 mg fresh weight of tumors were incubated at 25°C in 1 ml of feeding solution for different time intervals. Substrates (hexoses or amino acids) were always supplied at 1 mM concentration. The auxins or auxin antagonists were added from ethanolic stock solutions. The ethanol did never exceed 0.1% (v/v) of the feeding solution. Control incubations received the corresponding amount of pure ethanol. All feeding solutions contained 1 mM KH_2PO_4, 100 μM $CaCl_2$, and 50 mM KCl. The pH was adjusted to 6.0 with 1 N KOH after the substrate and

[1] Abbreviations: OMG, 3-*O*-methyl-D-glucose; 1-NAA, 1-naphthaleneacetic acid; 2-NAA, 2-naphthaleneacetic acid; 1-NOA, 1-naphthoxyacetic acid; 2-NOA, 2-naphthoxyacetic acid; PCIB, 2-(*p*-chlorophenoxy)-2-methylpropionic acid.

the auxin or auxin antagonist had been added. For the pH dependence of D-glucose uptake at higher buffer strength, Mes-Tris buffer was used; 3.3 KBq of labeled substrate was included in 1 ml of feeding solution.

The incubation was stopped by adding 5 ml of an ice cold 100 mM D-glucose solution when D-glucose or OMG were the substrates. When testing amino acid uptake, 10-mM solutions of the unlabeled amino acids were used. After rapid filtration on Whatman filter disks (Grade 3 MM) under suction (filtration time less than 5 s), the tissue was washed on the filter with another 5 ml of the corresponding solution.

Determination of Radioactivity. The tissue was homogenized with pestle and mortar in 1 ml of washing buffer. 500 μl aliquots of the resulting homogenate were thoroughly mixed with 3 ml of scintillant (Quickscint 212, Zinsser Analytical). Solubilization of the tissue with Soluene-350 (Packard) gave the same results. The radioactivity was determined in a Tricarb-3320 (Packard) liquid scintillation spectrophotometer. Quench correction was achieved by the channel ratio method. Counting times of samples were adjusted to accumulate ≧ 166 Bq.

Statistical Treatment of the Data. The data represent means of 2 or 3 independent experiments with tumors from different batches. Within one experiment all samples were taken in duplicates. The standard errors of the mean never exceeded ±10% and are omitted from the graphs for reasons of clarity.

RESULTS

Influence of Various Auxins and Auxin Antagonists on D-Glucose Uptake. After a rapid initial uptake for the first 10 min, the accumulation of D-glucose by 28-d-old tumors reached a constant rate which was maintained for more than 2 h (Fig. 1).

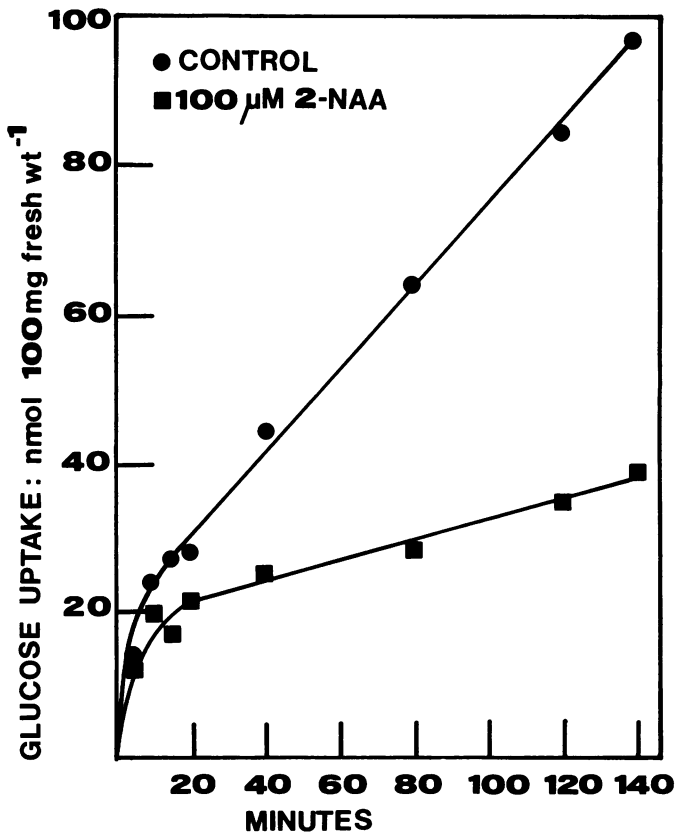

FIG. 1. Time course of D-glucose uptake by 28-d-old tumors in the presence and absence of the auxin antagonist 2-NAA. Glucose concentration is 1 mM.

Table I. *Influence of Different Auxins and Auxin Antagonists on the Uptake of D-glucose and 3-O-methyl-D-glucose, respectively*

Composition of feeding solution: 1 mM KH_2PO_4, 100 μM $CaCl_2$, 50 mM KCl, 1 mM substrate, 100 μM auxin or auxin antagonist, respectively, pH adjusted to 6.0 with 1 N KOH. Values are given ± SE with % stimulation or inhibition of control in brackets.

Compound	Uptake	
	D-glucose	3-*O*-methyl-D-glucose
	nmol/100 mg fresh wt·h	
Control	32.0 ± 2.9	31.4 ± 3.2
IAA	39.7 ± 3.5 (+24)	38.0 ± 2.5 (+21)
PCIB	17.6 ± 1.8 (−45)	18.9 ± 1.7 (−41)
1-NAA	26.2 ± 2.5 (−18)	24.9 ± 2.6 (−22)
2-NAA	6.7 ± 0.5 (−79)	5.9 ± 0.6 (−82)
1-NOA	19.2 ± 2.0 (−39)	17.5 ± 1.3 (−45)
2-NOA	28.8 ± 2.6 (−10)	29.1 ± 3.0 (−9)

In the presence of the auxin antagonist, 2-NAA, a drastic decrease of the uptake rate was observed with a lag period of less than 15 min (Fig. 1).

The effects of various auxins and auxin antagonists at 100 μM concentration on the uptake of D-glucose were compared (Table I). While IAA stimulated D-glucose uptake, all other compounds were inhibitory but to strikingly different degrees. The auxins 1-NAA and 2-NOA showed an inhibition of less than 20% while the auxin antagonists 2-NAA, 1-NOA, and PCIB reduced uptake by 40 to 80%.

Since the uptake of D-glucose might be influenced by its metabolism, a control experiment was performed with OMG (Table I). The data confirm that it is in fact the uptake which is modulated by the auxins and auxin antagonists, respectively.

Concentration Dependence of Uptake Inhibition by Auxins and Auxin Antagonists. The effects of auxins and auxin antagonists at different concentrations on the uptake of D-glucose during an incubation period of 20 to 80 min were compared (Fig. 2, A and B).

The antagonists 2-NAA, 1-NOA, and PCIB all showed a pronounced inhibition of D-glucose uptake at concentrations below 100 μM. For the strong inhibition by 2-NAA an apparent K_i value of 21 μM was obtained from a plot 1/per cent inhibition *versus* 1/[2-NAA] (Fig. 3).

Conversely, the auxins 1-NAA and 2-NOA caused little inhibition at low concentrations. A pronounced inhibitory effect became apparent at higher concentrations only. The natural auxin IAA was the only compound which slightly stimulated D-glucose uptake above control at concentrations ranging from 20 to 100 μM, but was equally inhibitory at higher concentrations.

Effects of pH and Buffer Concentration on D-Glucose Uptake. At a buffer concentration lower than 5 mM the uptake of D-glucose was independent of the pH of the feeding solution, whereas when increasing buffer concentration the uptake showed a pronounced pH dependence with an optimum at about 5 (Fig. 4, not all data shown). However, the inhibitory effect of 2-NAA could not be reversed by acid buffer. In the presence of 2-NAA, the buffer concentration *per se* did not change the uptake rate significantly at any pH value (Fig. 4).

Effects of Auxins and Auxin Antagonists on Amino Acid Uptake. The influence of IAA, 1-NAA, and 2-NAA on the uptake of several amino acids was compared (Table II). The uptake rates

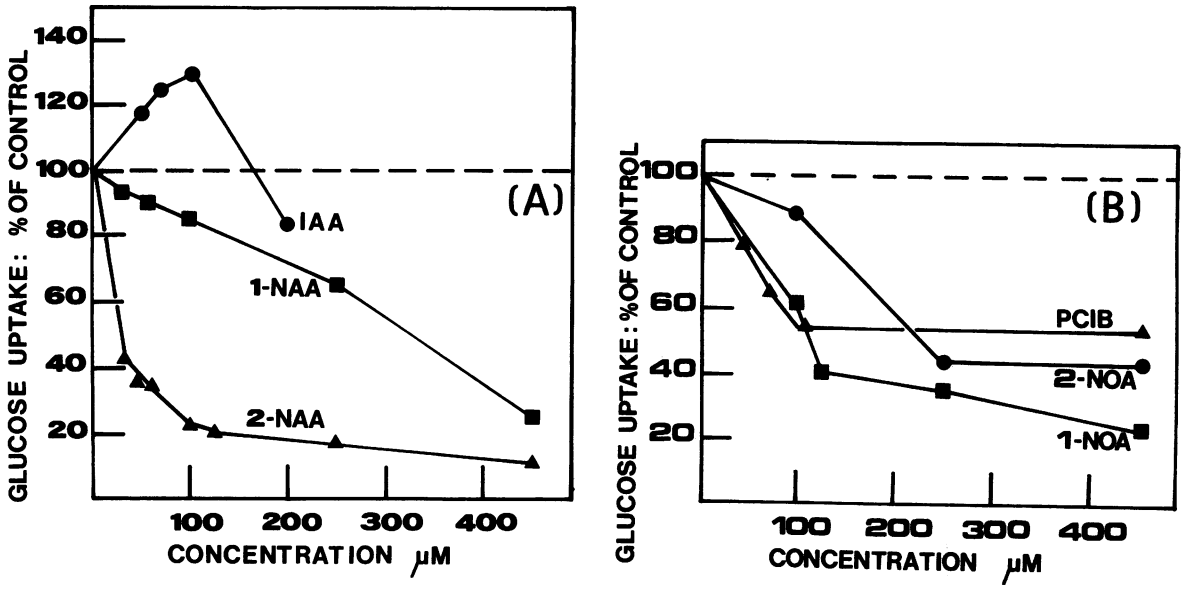

FIG. 2. A and B. D-Glucose uptake by 28-d-old tumors in the presence of different concentrations of the auxins IAA, 1-NAA, 2-NOA, and the auxin antagonists 1-NOA, 2-NAA, and PCIB, respectively. Glucose concentration is 1 mM. Control tissue shows an uptake rate of 32.0 nmol/g fresh weight·h.

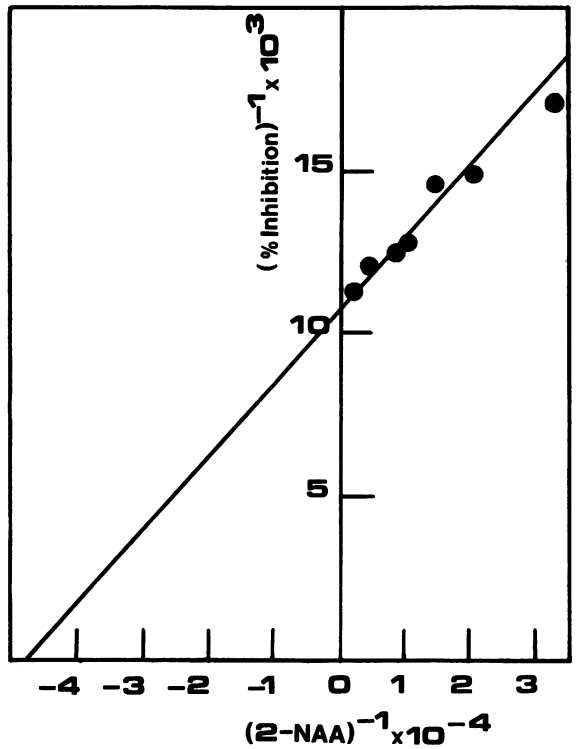

FIG. 3. Concentration dependence of the inhibition of D-glucose uptake by 2-NAA in a double reciprocal plot 1/per cent inhibition *versus* 1/[2-NAA]. The K_i value obtained is 21 μM.

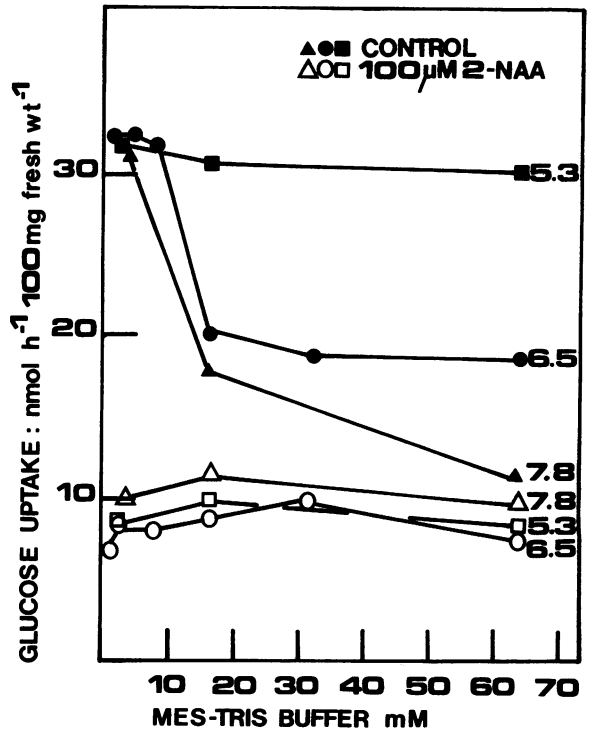

FIG. 4. pH-Dependence of D-glucose uptake by 28-d-old tumors at different buffer concentrations in the presence or absence of the auxin antagonist 2-NAA.

for Leu, Trp, Met, Lys, and Asp were all similarly modulated with a slight inhibition by 100 μM 1-NAA and a rather strong inhibition by 100 μM 2-NAA. However, the stimulatory effect of 100 μM IAA was rather weak or absent. Methionine showed a somewhat deviating pattern with a markedly decreased uptake in the presence of both IAA and 1-NAA. The absolute uptake of tryptophan was very high as compared to the other amino acids. Aspartic acid showed the lowest uptake rate of all amino acids tested.

DISCUSSION

Active Uptake of D-Glucose by Crown Gall Tumors. The uptake kinetics of D-glucose (Fig. 1) are biphasic with an initial rapid phase of about 10 min followed by a constant rate for more than 2 h. This result may be interpreted as a rapid diffusion

Table II. *Influence of IAA, 1-NAA, and 2-NAA on Uptake of Different Amino Acids by 28-d-old Tumors*

Composition of feeding solution: 1 mM KH_2PO_4, 100 μM $CaCl_2$, 50 mM KCl, 1 mM amino acid, 100 μM IAA, 1-NAA or 2-NAA; pH adjusted to 6.0 with 1 N KOH. Values are given ± SE with % stimulation or inhibition in brackets.

Amino Acid	Uptake			
	Control	IAA	1-NAA	2-NAA
	nmol/100 mg fresh wt·h			
Leu	12.5 ± 1.2	13.1 ± 1.2 (+5)	13.5 ± 1.4 (+8)	6.5 ± 0.5 (−48)
Met	11.4 ± 0.9	8.1 ± 0.7 (−29)	5.6 ± 0.6 (−49)	4.0 ± 0.3 (−65)
Trp	22.0 ± 1.8	19.6 ± 2.0 (−11)	15.7 ± 1.4 (−29)	7.9 ± 0.8 (−64)
Lys	13.6 ± 1.4	13.9 ± 1.4 (+2)	13.7 ± 1.2 (+1)	3.7 ± 0.4 (−73)
Asp	8.3 ± 0.9	7.2 ± 0.6 (−13)	6.0 ± 0.5 (−28)	0.9 ± 0.1 (−88)

of the substrate into the apoplastic space until equilibration at the cell surfaces followed by the active carrier mediated uptake which then becomes the rate limiting process. Elution kinetics from several plant tissues with labeled glucose, sucrose, and amino acids (T. Rausch, D. Butcher, K. Chamberlain, unpublished results) support this assumption. In systems without an extended apoplastic space like plant cell cultures, the initial diffusion component becomes negligible. In many other higher plant materials, D-glucose uptake at concentrations lower than 5 mM has been shown to be primarily carrier mediated (21). Further evidence for a carrier mediated uptake by the tumor tissue is provided by the expressed pH dependence at higher buffer concentrations (Fig. 4).

Influence of Auxins and Auxin Antagonists on Uptake of D-Glucose and Amino Acids. Many recent investigations suggest a hormonal control of the electrochemical proton gradient across the cell membrane in plants (7, 8, 17). However, the presence of high concentrations of endogenous hormones has excluded those objects from investigation which actively synthesize hormones to saturating levels. Only in systems with naturally or experimentally induced hormone deficiencies (7) may a positive reaction toward an exogenously supplied hormone be measured. The present study suggests that auxin antagonists may be successfully used to titrate the activity of endogenous IAA in crown gall tissue. Although an influence of auxin antagonists on the balance between free and bound IAA cannot be ruled out on the basis of the present data, such an effect seems unlikely in view of the almost immediate onset of the observed inhibition of nutrient uptake (Fig. 1).

A primary rapid effect of IAA is the stimulation of proton efflux in many tissues (7). As the active uptake of hexoses by plant cells proceeds in a symport with protons (21), the uptake of D-glucose was chosen to measure hormone action. When accurately evaluating the influence of IAA or its antagonists on the uptake of D-glucose, several experimental conditions have to be fulfilled: (a) The glucose concentration must be so low as to reduce any diffusion of the solute into the cells to a minimum; (b) the buffer capacity of the feeding solution must be high enough to neutralize the acidic auxins or antagonists but low enough so that it does not interfere with an increased proton excretion from the cells as a result of hormone action; (c) identical experiments with a nonmetabolized derivative taken up by the same carrier system have to be included to differentiate between a modulation of uptake and metabolism. All these conditions are met when D-glucose is supplied at 1 mM and the buffer concentration is kept below 5 mM (Fig. 4). The comparison with OMG shows that it is, in fact, the uptake which is measured and modified by the auxins and auxin antagonists (Table I).

The auxin antagonists used in the present study, namely 1-NOA, 2-NAA, and PCIB, have been shown to efficiently and specifically compete with IAA for auxin binding sites *in vitro* (9, 11, 13, 22). This holds for the binding sites on the plasmalemma, the tonoplast, and the ER. Therefore, the strong inhibition of glucose and amino acid uptake by 2-NAA and other auxin antagonists (Fig. 2, A and B; Table II) may be attributed to a specific competition with the endogenous IAA for common binding sites thus neutralizing the stimulating effect of endogenous IAA on the electrochemical proton gradient (7). The calculated K_i value of 21 μM for 2-NAA compares favorably with the inhibition constants of this compound for the auxin binding sites on maize coleoptile membranes *in vitro* (11, 22). The auxin antagonists 1-NOA and PCIB equally reach their maximum inhibition at about 100 μM (Fig. 2, A and B).

The inhibitory effect of the auxins 1-NAA and 2-NOA at higher concentrations is in agreement with the demonstrated inhibition by IAA at supraoptimum levels (Fig. 2, A and B). The observation that only IAA is able to stimulate glucose uptake above control while 1-NAA and 2-NOA both slightly inhibit even below 100 μM may be interpreted as the competition of these compounds with endogenous IAA. Especially with the strong auxin 1-NAA, it could be that supraoptimum levels are reached at even lower concentrations than found for IAA.

The strong inhibition of glucose uptake by 2-NAA and 1-NOA is certainly no unspecific inhibitory effect, as these compounds are able to stimulate glucose uptake significantly in tissues with supraoptimum IAA levels like primary roots or mesocotyl from maize (T. Rausch, W. Hilgenberg, unpublished results).

The modulating effect of added IAA on the electrochemical proton gradient in auxin-sensitive plant tissues is well documented (7, 18). In IAA deficient tissues, a hyperpolarization of the membrane potential (18) and an increased proton excretion (7) are observed after the addition of IAA. In tissues with supraoptimum IAA levels the same effects may be obtained with the auxin antagonist PCIB (19). IAA, when added exogenously to maize coleoptiles, may stimulate glucose and amino acid uptake by about 20% (8, 12). Although the pH difference across the plasmalemma is certainly a primary factor regulated by IAA, it is not the only mechanism by which IAA stimulates the solute uptake. Otherwise it should have been possible to reverse the inhibition of D-glucose uptake by 2-NAA with acid buffer at appropriate concentrations. However, this cannot be achieved (Fig. 4). As IAA may stimulate both the pH gradient and the membrane potential (18), it is possible that the antagonist interferes with both. In this case a simple reversal of the antagonist-mediated inhibition by lowering the pH would not be expected. Furthermore, it is too premature to speculate about other events in the cytoplasm which are under the control of IAA, but there are reasons to assume that IAA affects the Ca^{2+} distribution (16) which in turn has an influence on the fusion of Golgi vesicles with the plasmalemma and the exchange of membrane components between both (6). Glucose carrier proteins as well as ATPase precursors could be among the exchanged components.

The Role of IAA for the Development and Maintenance of a Metabolic Sink in Crown Gall Tissue. The extremely rapid growth of primary crown gall tumor cells depends almost exclusively on an extensive and adequate supply of nutrients from the surrounding host tissue. The developing tumor attracts these nutrients presumably by a high electrochemical proton gradient which is drastically enhanced by the endogenous IAA. For that reason, IAA serves a central role for tumor growth and its high concentration in crown gall tissue is probably an indication of its involvement in tumor development (3, 20, 25, 26). The drastic

IAA-mediated increase in hexose uptake could be the essential factor for the development of a metabolic sink. When the uptake of glucose in the presence of 100 μM 2-NAA is compared with the uptake in the control tissue, it follows that the endogenous IAA increases the uptake rate five-fold (Fig. 2, A and B).

Using the specific effects of auxin antagonists, the influence of endogenous IAA during different stages of tumor development may now be assessed. The rapidly growing 28-d-old primary tumor tissue used in our investigation is obviously not completely saturated with endogenous IAA (Fig. 2A). This shows that the IAA receptors are not a limiting factor. The possible range of an IAA-mediated increase of D-glucose uptake is determined by, (a) the number of D-glucose carrier proteins, (b) the membrane potential, (c) the pH gradient across the plasmalemma, (d) the number of IAA receptors, (e) the concentration of IAA itself and, possibly, other yet unknown factors. The method developed permits an estimation of the resulting overall effect and gives some ideas as to the mechanism involved. Detailed studies on the D-glucose carrier protein and the IAA receptors are under way in our laboratory.

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LITERATURE CITED

1. AKIYOSHI DE, RO MORRIS, R HINZ, BS MISCHKE, T KOSUGE, DJ GARFINKEL, MP GORDON, EW NESTER 1983 Cytokinin/auxin balance in crown gall tumors is regulated by specific loci in the T-DNA. Proc Natl Acad Sci USA 80: 407–411
2. ANAND VK, GT HEBERLEIN 1977 Crown gall tumorgenesis in potato tuber tissue. Am J Bot 64: 153–158
3. ATSUMI S 1980 Relation between auxin autotrophy and tryptophan content in sunflower crown gall cells in culture. Plant Cell Physiol 21: 1031–1039
4. ATSUMI S 1980 Studies on the production of indole-3-acetic acid with auxin-heterotrophic mutants derived from cultured crown gall cells. Plant Cell Physiol 21: 1227–1232
5. ATSUMI S, T HAYASHI 1978 The relationship between auxin concentration, auxin protection, and auxin destruction in crown gall cells cultured in vitro. Plant Cell Physiol 19: 1391–1397
6. BAYDOUN EA, DH NORTHCOTE 1980 Measurement and characteristics of fusion of isolated membrane fractions from maize root tips. J Cell Sci 45: 169–186
7. CLELAND RE 1982 The mechanism of auxin-induced proton efflux. *In* PF Wareing, ed, Plant Growth Substances. Academic Press, New York, pp 23–31
8. COLOMBO R, MI ME MICHELIS, P LADO 1978 3-*O*-methylglucose uptake stimulation by auxin and by fusicoccin in plant materials and its relationship with proton extrusion. Planta 138: 249–256
9. CROSS JW, WR BRIGGS, U DOHRMANN, PM RAY 1978 Auxin receptors of maize coleoptile membranes do not have ATP-ase activity. Plant Physiol 61: 581–584
10. DESPEGHEL JP, S DELROT 1983 Energetics of amino acid uptake by *Vicia faba* leaf tissues. Plant Physiol 71: 1–6
11. DOHRMANN U, R HERTEL, H KOWALIK 1978 Properties of auxin binding in different subcellular fractions from maize coleoptiles. Planta 140: 97–106
12. ETHERTON B, B RUBINSTEIN 1978 Evidence for amino acid H^+ co-transport in oat coleoptiles. Plant Physiol 61: 933–937
13. JACOBS M, R HERTEL 1978 Auxin binding to subcellular fractions from Cucurbita hypocotyls: In vitro evidence for an auxin transport carrier. Planta 142: 1–10
14. JUNG KD, U LÜTTGE, E FISCHER 1982 Uptake of neutral and acidic amino acids by Lemna gibba correlated with the H^+-electrochemical gradient at the plasmalemma. Physiol Plant 55: 351–355
15. KINRAIDE TB, B ETHERTON 1980 Electrical evidence for different mechanisms of uptake for basic, neutral and acidic amino acids in oat coleoptiles. Plant Physiol 65: 1085–1089
16. KUBOWICZ BD, LN VANDERHOEF, JB HANSON 1982 ATP-dependent calcium transport in plasmalemma preparations from soybean hypocotyls. Plant Physiol 69: 187–191
17. MARRE E 1982 Hormonal regulation of transport: data and perspectives. *In* PF Wareing, ed, Plant Growth Substances. Academic Press, New York, pp 407–417
18. MIZUNO A, K KATOU, H OKAMOTO 1980 Structure and function of the elongation sink in the stems of higher plants. I. Effects of anoxia and IAA on the growth rate and the spatially separate electrogenic ion pumps. Plant Cell Physiol 21: 395–403
19. MOLONEY MM, PE PILET, MT MARRE, G ROMANI 1982 Transmembrane electrical potentials in growing maize roots. Planta 156: 407–412
20. MOUSDALE DMA 1982 Endogenous indolyl-3-acetic acid and the growth of auxin-dependent and auxin-autotrophic (crown gall) plant tissue cultures. Biochem Physiol Pflanz 177: 9–17
21. NOVACKY A, CI ULLRICH-EBERIUS, U LÜTTGE 1980 pH and membrane potential changes during glucose uptake in Lemna gibba G 1 and their response to light. Planta 149: 321–326
22. RAY PM, U DOHRMANN, R HERTEL 1977 Characterization of naphthaleneacetic acid binding to receptor sites on the cellular membranes of maize coleoptile tissue. Plant Physiol 59: 357–364
23. STONIER T 1969 Studies on auxin protectors. VII. Association of auxin protectors with crown gall development in sunflower stems. Plant Physiol 44: 1169–1174
24. STONIER T 1970 The role of auxin protectors in autonomous growth. Colloques internationaux C.N.R.S. Paris 193: 423–435
25. WEILER EW 1981 Radioimmunoassay for pmol-quantities of indole-3-acetic acid for use with highly stable (^{125}I)- and (^{3}H)IAA derivatives as radiotracers. Planta 153: 319–325
26. WEILER EW, K SPANIER 1981 Phytohormones in the formation of crown gall tumors. Planta 153: 326–337