INCREASED LEVELS OF PISATIN AND PHENYLALANINE AMMONIA LYASE ACTIVITY IN PISUM SATIVUM TREATED WITH ANTIHISTAMINIC, ANTIVIRAL, ANTIMALARIAL, TRANQUILIZING, OR OTHER DRUGS.*

Lee A. Hadwiger

Department of Plant Pathology, Washington State University, Pullman, Washington 99163.

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SUMMARY

The de novo synthesis of the phytoalexin pisatin can be initiated in excised pea pod tissue by the drugs tilorone, chloroquine, imipramine, quinacrine, and other structurally analogous compounds. Some such drugs can enhance the level of phenylalanine ammonia lyase (PAL) activity up to 17 times that in control tissue. The enhancement of both responses is accompanied by differential incorporations of leucine into soluble proteins.

INTRODUCTION

Pisatin, an isoflavonoid with antifungal properties, is produced in pea tissues when challenged by certain plant pathogenic fungi (1). Disease resistance is believed to be associated with the induction of pisatin synthesis (1) and/or the accompanying changes in protein synthesis (2) induced in pea tissue by plant pathogens unable to incite pea diseases. Certain fungal metabolites (1,3), synthetic compounds (1,4,5) or U.V. light (6) can trigger the de novo production of pisatin (6) in excised pea pod tissue in the absence of a parasite. Pisatin synthesis depends on the synthesis of RNA and protein and usually is accompanied by dramatic increases (5) in phenylalanine ammonia lyase (PAL) [a key enzyme in pisatin biosynthesis (7)] activity as well as characteristic changes in the rate of RNA and protein synthesis (4,8).

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It has been established (5) that many compounds which can intercalate into DNA, or possess basic groups (or side chains) capable of ionic binding to the phosphate groups of DNA can also trigger the de novo synthesis of pisatin. This report demonstrates that clinically important drugs with such capabilities are also inducers. Concomitantly a mode of action common to medicinally diverse but chemically related drugs is demonstrated.

MATERIALS AND METHODS

Immature pea (Pisum sativum L) pods (2 cm) were harvested while still enclosed in the blossoms. Samples (1 g) of the separated pod halves were placed in sterile petri dishes. Drugs were administered to the exposed endocarp as aqueous solutions. Those drugs with low water solubility were alternately applied in pure corn oil. The pods were then incubated in the dark for 18 hours before the extraction of phenylalanine ammonia lyase or 24 hours before the extraction of pisatin. The phenylalanine ammonia lyase assay, pisatin quantitation and protein labeling techniques have been described (3,5,8). The rate of protein synthesis in pea tissue treated with drugs was compared with the rate in tissue treated with H_2O by measuring the incorporation of L-leucine-U-^{14}C and L-leucine-4,5-^{3}H into the respective proteins. For uniformity, tissues were compared 9 hours subsequent to treatment, since optimal increases in rate of protein synthesis in drug-induced tissue have been shown to occur 8 to 10 hours subsequent to inducer application (8).

RESULTS AND DISCUSSION

Phenylalanine ammonia lyase activity and pisatin production in peas is dramatically increased by tilorone , chloroquine, imipramine, quinacrine (Table 1) or other chemically analogous drugs. Histaminic and antimalarial drugs are generally the most effective in increasing the levels of both phenylalanine ammonia lyase and pisatin. The increased synthesis of pisatin is usually, but not always, proportional to the increased level of phenylalanine ammonia lyase.

Table 1. The effect of various drugs on the levels of phenylalanine ammonia lyase and pisatin in pea pod cells.[a]

Drug	Conc. (mg/ml)	PAL % of Control[c]	Pisatin (μg/g)
H_2O		100	not detectable
anticonvulsant			
carbamazepine	1.0	132	0
antidepressant			
amitriptyline	1.0	504	44
amitriptyline	.1	744	68
imipramine	.5	739	49
nortriptyline	1.0	379	27
nortriptyline	.1	431	23
protriptyline	1.0	384	30
	.1	271	-
anticholinergic			
propantheline bromide	1.0	714	142
propantheline bromide	.1	265	-
antileukemial agent			
pseudourea	.1	377	15
antipruritic			
trimeprazine	1.0	718	42
trimeprazine	.1	235	24
antispasmodic			
methixene	.5	626	56
methixene	.1	1010	82
antitussive			
dimethoxanate	1.0	884	22
antiviral agent			
tilorone	1.0	450	50
antimalarial agent			
chloroquine	1.0	377	26
hydroxy-chloroquine	1.0	340	10
pamaquine	1.0	1731	195
pamaquine	.1	334	26
primaquine	1.0	1100	135
primaquine	.1	225	64

Drug	Conc. (mg/ml)	PAL % of Control	Pisatin (µg/g)
quinacrine	1.0	1416	75
quinine	1.0	1616	102
antihistaminic			
bromodiphen-hydramine	1.0	1009	29
carbinoxamine	1.0	831	77
chlorpheniramine	1.0	1617	185
chlorpheniramine	.5	578	-
isothipendyl	1.0	1225	170
phenindamine	1.0	1215	135
pyrathiazine	1.0	807	7
triprolidine	1.0	698	32
tranquilizer			
carphenazine	1.0	380	0
chlorpromazine sulfoxide	1.0	1108	96
chlorprothixene	1.0	704	54
perphenazine	1.0	268	36
piperacetazine	1.0	578	23
propiomazine	1.0	702	23
thiopropazate	1.0	300	17
thioridazine	1.0	535	31

[a]The effects of several of these drugs have been reported previously and were included in this table for comparative purposes. Values represent averages obtained from 3-5 separate experiments; all treatments could not be compared within a single experiment because of limited plant material.

[b]One ml aqueous solution of each drug was applied to the pods immediately prior to being incubated in the dark at 22° C.

[c]Phenylalanine ammonia lyase (PAL) activity of the water induced control averaged 125 mµmoles cinnamic acid /g pod/hr at 37° C. PAL was extracted and assayed 18 hours after inducer application.

[d]Pisatin was extracted 24 hours after drug application.

The compounds in Table 1 are medicinally diverse, however all of the compounds assayed were selected on the basis of structural components previously established (5,8) to be beneficial for induction. The structures of these compounds are presented in Fig. 1. The majority of these compounds have a tricyclic nucleus with aliphatic substitutions on the center ring, i.e., ring position 10 of chlorpromazine or position 4 of quinoline. The aliphatic

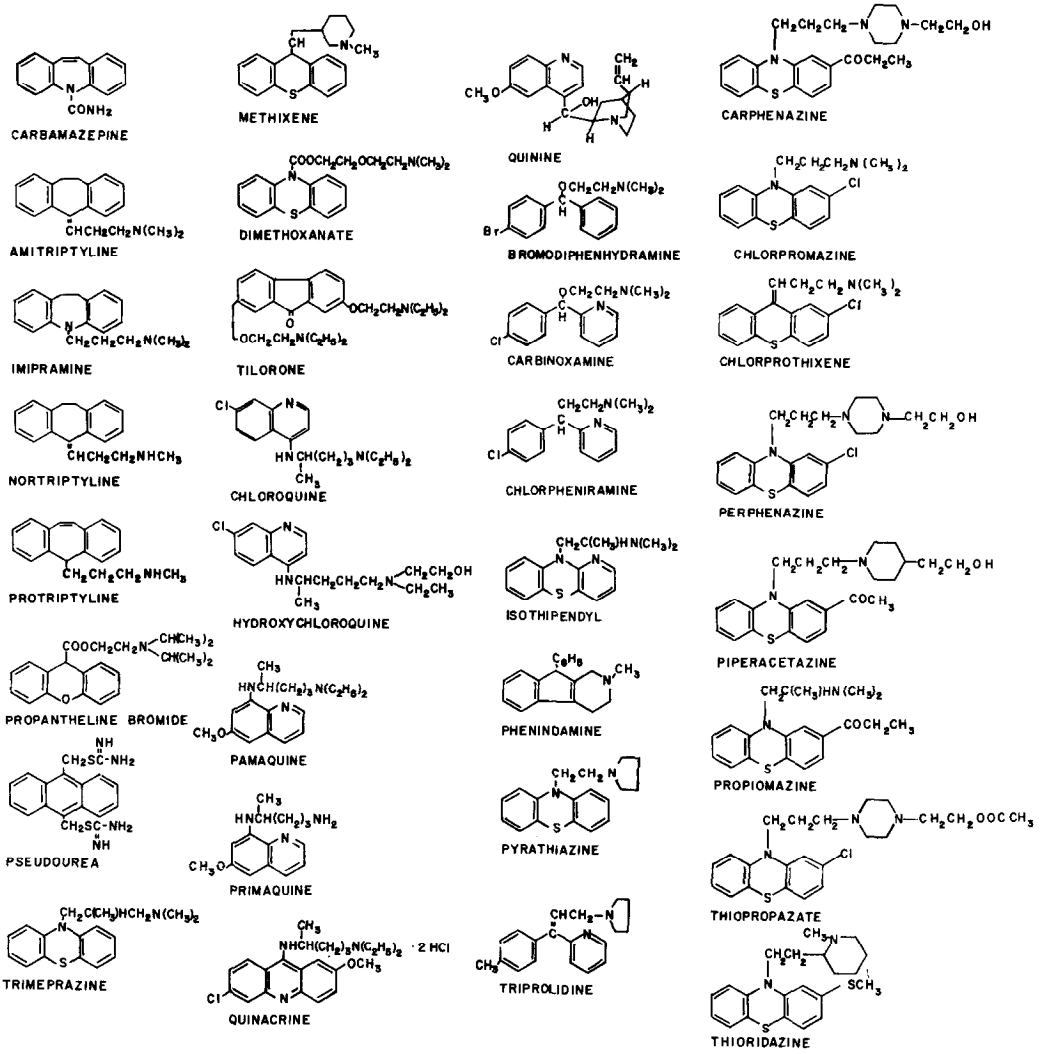

Fig. 1. Structural formulas for compounds listed in Table 1.

side chains of many of the compounds terminate with dimethylamino or diethylamino groups. These groups contribute significantly to the pisatin inducing potential (5). Most of the inducing compounds also contain a nitrogen atom in one of the rings.

Tilorone, chlorpheniramine and pseudourea [2,2'-(9,10-anthrylenedimethylene) bis (2-thiopseudourea)] characteristically change (Fig. 2) the rates at which leucine is incorporated into the various protein fractions of peas. Apparently the action of the drug is not limited to increasing

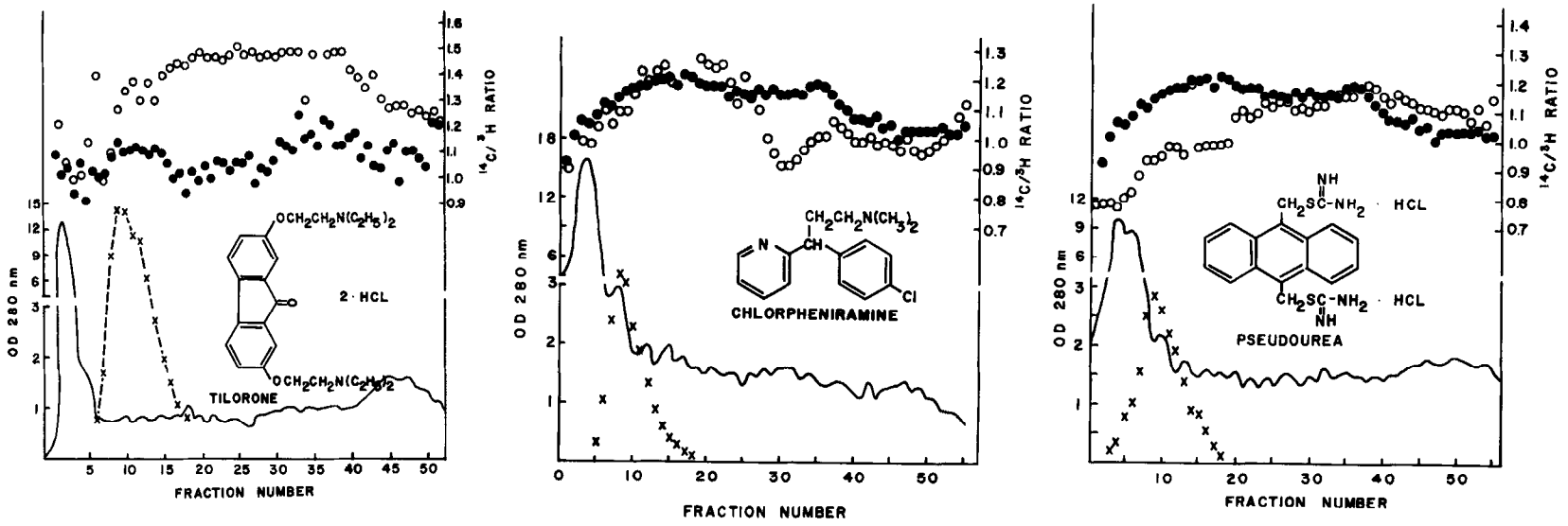

Fig. 2. Relative rate of incorporation of radioactively labeled leucine into soluble proteins of drug-treated pea pods versus incorporation into water-treated tissue. One 3-g sample of drug-treated pods was labeled with 3 μc L-leucine-^{14}C 9 hr subsequent to drug application. A second sample of water-treated pods was simultaneously labeled with 30 μc L-leucine-4,5^{3}H. The two samples were combined and the soluble proteins extracted after a 45-min pulse. The protein was dialyzed, concentrated and subsequently fractionated on Sephadex G-200 as described previously (7). The ratios (corrected for counting efficiency and relative counts administered) of leucine-^{14}C to leucine-^{3}H in these experiments are indicated by the open circles. The closed circles indicate the ratios of dual labeled control experiments obtained by pulse labeling two water-treated samples (pods were from the same harvest). The solid line indicates optical density at 280 nm. The x's indicate the fractions containing PAL activity.

pisatin or pisatin synthesizing enzymes, since changes in the relative rate of incorporation occur in most of the protein fractions. Although each inducer stimulates pisatin synthesis and increases the activity of PAL many fold, compounds such as chlorpheniramine can decrease the net rate of leucine incorporation, even in those fractions containing increased levels of phenylalanine ammonia lyase activity. The drug induced increases in phenylalanine ammonia lyase are apparently dependent on RNA and protein synthesis since they are inhibited by the simultaneous application of 6-methylpurine or cycloheximide. In pea pod tissue 6-methylpurine (0.1 mg/ml) inhibits RNA synthesis 63% within 1 hour and cycloheximide (0.01 mg/ml) inhibits protein synthesis 90% within 1-2 hours after application (9).

The effective concentrations of the drugs used in this study are quite substantial when compared to the concentrations utilized in mammalian systems. However, radioautographic and other histological studies of compound penetration in this pea pod system indicate that only a small percentage of molecules penetrate the cell within the crucial hour following inducer application (5). This may partially explain the necessity for relatively high drug concentrations.

The drug-induced changes in the pattern of proteins synthesized and in phenylalanine ammonia lyase activity probably reflect gross alterations in the regulation of pea cells. The pisatin-inducing drugs also differentially effect seed germination and growth in intact pea plants. Some such drugs promote stunting, distortion and/or unilateral elongation of roots and shoots following seed germination.

The intracellular target for these drugs has not been resolved. However, a number of other compounds which induce pisatin and phenylalanine ammonia lyase activity are believed to bind specifically, or in some other way associate, with double-stranded DNA. These compounds include: (i) alkylating agents such as nitrosoquanidine, and mitomycin C (8), (ii) polyamines, such as spermine and spermidine (10), (iii) polypeptides such as

poly-L-lysine, poly-L-arginine, poly-L-ornithine and protamine (4,10),(iv) base analogs such as 8 azaguanine (8), and (v) DNA intercalating compounds such as ethidium bromide, nogalamycin, and actinomycin D (2,5). The induction of these pea responses has been proposed to result from specific changes in the in vivo conformation of DNA (8). Evidence in support of this hypothesis and in relation to DNA as the cellular target for these inducing compounds has been presented in detail previously (5,8).

More recently Waring (11) has conclusively demonstrated that these DNA intercalating compounds induce local uncoiling of the double helical DNA of ϕX 174. However, drugs such as chlorpromazine and primaquine, previously categorized as intercalators, do not promote uncoiling and are now believed to interact with DNA by mechanisms other than intercalation. These latter compounds and many of the other PAL inducing drugs have basic side chains attached to the ring system which are capable of ionic binding to one or both strands of DNA (5).

Many questions remain to be answered as to the exact mode by which these drugs act in pea tissue. Nevertheless, this system has enabled us to pinpoint the structural groups of pisatin and PAL inducers which are beneficial to the induction process. Since certain of these structural groups are inherent to compounds released by microorganisms, the mode by which plant pathogenic microorganisms incite pisatin production and other gross changes (12) in the physiology of the plant host becomes more lucid.

The ability of certain drugs to induce responses, in addition to the phytoalexin promoting response, provides some promise for chemically manipulating disease resistance in plant tissue. In turn, this analysis of the gross physiological changes caused by these drugs in plant tissue may provide further insight into the basis of their manifold effects in mammalial tissues.

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