Acrolein Induces Differentiation of Infection Structures in the Wheat Stem Rust Fungus

Abstract. *A chemical factor from wheat stem rust uredospores that induces infection structure formation has been identified as acrolein (2-propenal). This compound is the active component of distillates from uredospore extracts previously shown to induce infection structure formation.*

In many plant-pathogenic fungi, hyphae on the host surface develop specialized structures which are involved in penetration of the host. In germinating uredospores of rust fungi, these infection structures include appressorium, penetration peg, vesicle, and infection hyphae. Nuclear and cell divisions occur during the differentiation process (*1*). This sequence of structures is apparently initiated by the contact of the germ tube with a guard cell of a stoma (*2*). Although the regulatory mechanisms are unknown, homologous structures can be induced in the absence of the host by substitute triggers (*2*). In several studies, recently reviewed by Allen (*3*), the experimental conditions necessary for differentiation in vitro were reported. For example, germinating uredospores of *Puccinia graminis* f. sp. *tritici*, floating on aqueous solutions or in suspension, produced infection structures in response to heat shock or to distillates from uredospore extracts (*4*). The latter finding suggested that a volatile chemical agent was present which initiated infection structure formation and that this agent might be an intermediary in the contact-triggered differentiation (*3*). The purpose of the work reported here was to isolate and identify the chemical inducer of differentiation from uredospores.

Uredospores of *P. graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., race 56, were obtained from infected wheat (*Triticum aestivum* cv. 'Little Club') and stored as described previously (*5*). Active material was extracted by stirring uredospores in water (20 g of spores per liter). After filtration through sintered glass to remove spores, the extract was distilled at atmospheric pressure through a 60-cm Vigreux column with the condenser at 1°C. The distillate and the fractions from subsequent purification steps were tested under standard assay conditions in closed vessels at 20°C in darkness. The center wells of 6-ml Pyrex micro-Conway diffusion dishes contained spores floated on 0.2 ml of 5 m*M* potassium phosphate buffer, *p*H 7.0. The outer wells contained an aqueous dilution series of the fractions. To increase the percentage germination, 1-nonanol (10 nmole per outer well) was added occasionally when spore lots showed low or erratic germination (*6*). Nonanol did not affect the differentiation-inducing activity of fractions. Control spores under these standard conditions germinated with long, undifferentiated, cylindrical germ tubes.

The stimulatory material was in the first 10 percent of the distillate and was initially concentrated by redistilling the active portion. Purification and isolation of the stimulant were achieved by reversed-phase high-performance liquid chromatography (HPLC), using water as the mobile phase. Eighty-five percent of the stimulatory activity was recovered in a fraction eluted at 9 minutes (*7*). In this fraction and in unpurified preparations the stimulatory activity could be removed by reagents which react with carbonyl groups. Consequently, a 2,4-dinitrophenylhydrazone derivative of the unknown chemical was prepared. The mass spectrum of the derivative was identical to that of the dinitrophenylhydrazone of acrolein (*8*). The ultraviolet spectrum of the active fraction (in water) from HPLC revealed a single absorption maximum at 209 nm. The spectra of this fraction and authentic acrolein were identical. Gas chromatographic analysis of the same fraction revealed a single peak coincident with that of acrolein (*9*). The concentration of acrolein in the active fraction from HPLC was estimated by using an extinction coefficient $E^{1\%}_{209} = 2060$. In a standard assay, both the compound from the spores and the authentic acrolein induced infection structures in 50 to 80 percent of germinating uredospores at 0.4 ± 0.1 nmole (*10*).

Table 1. Unsaturated aliphatic aldehydes and ketones which induce infection structure formation in *Puccinia graminis* f. sp. *tritici*.

Compound	Amount* (nmole)
2-Propenal $CH_2=CHCHO$	0.4 ± 0.1
3-Buten-2-one $CH_2=C(CH_3)CHO$	3.5 ± 1.5
1-Penten-3-one $CH_2=CHCOCH_2CH_3$	4.5 ± 1.5
2-Methyl propenal $CH_2=C(CH_3)CHO$	25 ± 15
3-Methyl-3-buten-2-one $CH_2=C(CH_3)COCH_3$	30 ± 15
trans-3-Penten-2-one $CH_3CH=CHCOCH_3$	250 ± 150

*Amount of compound which induces infection structures in 50 to 80 percent of germinating uredospores in the standard assay.

Structure-activity tests of over 60 compounds with varying structural similarities to acrolein indicated that differentiation was generally induced by short-chain aliphatic carbonyl compounds with a conjugated double bond (Table 1). Saturated aliphatic aldehydes and ketones, saturated and unsaturated aliphatic alcohols, and phenylacrolein (cinnamaldehyde) were not active (*11*).

A volatile compound may be involved in the "heat shock" induction of infection structures. Germinating spores differentiate only if heat shock is administered in closed vessels (*12*). Unidentified volatile compounds from wheat leaves also cause infection structure formation (*13*). Acrolein, as an inducer of differentiation, may be a normal intermediary or a coincidental factor. This compound could also form under aerobic conditions in aqueous solutions of amino acids and polyamines (*14*). Although its role in vivo, if any, is not known, acrolein has a definite morphogenetic effect.

Many volatile and nonvolatile extracellular products of eukaryotic microbes have morphogenetic effects in vitro and may or may not have a role in developmental processes in vivo (*15*). Experimental use of acrolein should facilitate work toward understanding how the physiological response of germinating uredospores to environmental signals results in either the maintenance of cylindrical undifferentiated germ tube growth or the induction of infection structures. Further, it would be interesting to compare the mode of action of acrolein as a stimulatory compound with a possible regulatory role in morphogenesis in the wheat stem rust fungus with its mode of action as a cytotoxic and mutagenic agent in other systems (*16*).

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10. Because acrolein is effective over a narrow concentration range and because of inherent variability in the bioassay (in number, quality, and germination percentage of spores), preliminary experiments were often necessary to obtain the effective concentration with a particular lot of spores.
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Dopaminergic Inhibition of Adrenergic Neurotransmission as a Model for Studies on Dopamine Receptor Mechanisms

Abstract. *Dopamine and apomorphine produced concentration-dependent inhibition of adrenergic neurotransmission in the isolated, perfused, rabbit ear artery. The inhibitory action of both dopamine and apomorphine was competitively antagonized by haloperidol and several other antipsychotic drugs. The calculated affinities of these drugs for the dopaminergic receptor correlate closely with both the pharmacological potencies of these drugs in vivo and their reported potencies as inhibitors of [^{3}H]haloperidol binding to "dopamine receptors" in brain homogenates.*

The neurotransmitter action of dopamine is mediated by distinct dopamine receptors (*1, 2*). Most antipsychotic drugs are thought to exert their therapeutic actions and to produce extrapyramidal side effects by blocking dopamine receptors (*3*). However, the precise pharmacological characteristics of these receptors have been only poorly defined. Indeed, it has been suggested that there may be more than one type of dopamine receptor in the brain (*4*).

Most of our knowledge of the characteristics of the different types of adrenergic and cholinergic receptors stems from studies on peripheral tissues. Although there are several peripheral dopaminergic receptor systems, none of these have been found suitable for quantitation of the action of dopaminergic agonists and antagonists. Recently, several investigators have demonstrated that some postganglionic sympathetic nerve terminals possess dopaminergic receptors which, when activated, mediate inhibition of nerve-evoked norepinephrine release (*5*). We now report that the inhibitory action of dopaminergic agonists on adrenergic neurotransmission in the isolated, perfused, rabbit ear artery provides an excellent experimental model for quantitative studies on dopaminergic receptor mechanisms.

The preparation of the isolated rabbit ear artery and the perfusion techniques used have been described (*6*). In brief, the artery, mounted in a narrow-bore muscle chamber, was simultaneously perfused intraluminally and superfused extraluminally. Both perfusion and superfusion were at constant flow rates (about 2 ml/min for each), so that the inflow perfusion pressure, recorded with a transducer and Physiograph (E & M Instrument), monitored the degree of vasoconstriction. The perfusion and superfusion fluid was oxygenated Krebs-bicarbonate solution maintained at 35°C. The periarterial adrenergic nerves were excited by field stimulation with square-wave pulses of 0.7-msec duration at supramaximal voltage (60 to 70 volts), applied through two platinum electrodes mounted in the muscle chamber. Except for a few experiments in which norepinephrine was administered intraluminally to produce vasoconstriction, all drugs were administered extraluminally by way of the inflowing superfusion fluid.

Dopamine, at concentrations ranging from 3 to 1000 nM, produced concentration-dependent inhibition of the constrictor response of the rabbit ear artery to brief intermittent periods of nerve stimulation (Fig. 1A). At high concentrations, dopamine also produced vasoconstriction. The vasoconstrictor response to dopamine was blocked by phentolamine and other α-adrenergic antagonists. Both the inhibitory effect on adrenergic neurotransmission and the vasoconstrictor effect of dopamine were markedly potentiated by cocaine, which acts as an inhibitor of neuronal dopamine uptake and thus slows the process of dopamine inactivation. All of the results described in this report were obtained in the presence of 3 μM cocaine.

Propranolol, a β-adrenergic antagonist, had no effect on dopamine-induced inhibition of neurotransmission. Dopamine did not inhibit the vasoconstrictor response to intra- or extraluminally administered norepinephrine. Thus the inhibition of neurotransmission by dopamine appears to result from an interference with the process by which nerve stimulation causes release of norepinephrine.

Apomorphine, which acts as a dopaminergic agonist in behavioral tests, also produced concentration-dependent inhibition of neurotransmission in the ear artery (Fig. 1B). Apomorphine and dopamine were equipotent as inhibitors of neurotransmission; the concentration-effect curves for these two agonists were essentially superimposable over the entire concentration range tested (Fig. 1C). The concentration required to produce 50 percent inhibition of the response to nerve stimulation was 37 ± 6 nM for dopamine and 44 ± 6 nM for apomorphine. Haloperidol antagonized the inhibitory effect of both dopamine and apomorphine. The antagonism was competitive; that is, haloperidol caused a parallel shift to the right of the log concentration-effect curves for the agonists (Fig. 1D).

The onset of antagonism was slow; at the concentrations used for these experiments, haloperidol had to be administered for approximately 1 hour in order to produce maximal antagonism of the inhibitory action of dopamine. The washing out of the haloperidol required several hours, probably because of the highly lipophilic nature of this agent. Several other antipsychotic drugs produced similar competitive antagonism of dopaminergic inhibition of neurotransmission. Based on experimental data of the type shown in Fig. 1D, we calculated the dissociation constants (K_B) for the receptor-antagonist complex for a series of antipsychotic drugs including the butyrophenones (spiroperidol, droperidol and haloperidol); the diphenylbutylpiperidines (pimozide and penfluridol), and a phenothiazine (perphenazine) (Table 1). (+)-Butaclamol acted as a potent dopaminergic antagonist, whereas (−)-butaclamol did not possess any antagonist activity at con-

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