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MiniReview

Origins and significance of ergot alkaloid diversity in fungi

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Abstract

Ergot alkaloids are a diverse family of indole-derived mycotoxins that collectively have activities against a variety of organisms including bacteria, nematodes, insects, and mammals. Different fungi accumulate different, often characteristic, profiles of ergot alkaloids rather than a single pathway end product. These ergot alkaloid profiles result from inefficiency in the pathway leading to accumulation of certain intermediates or diversion of intermediates into shunts along the pathway. The inefficiency generating these ergot alkaloid profiles may have been selected for as a means of accumulating a diversity of ergot alkaloids, potentially contributing in different ways to benefit the producing fungus.

Keywords: Ergot alkaloids; Clavines; Mycotoxin; *Neotyphodium*; *Claviceps*; *Aspergillus fumigatus*

1. Introduction

The ergot alkaloids are a complex family of mycotoxins derived from prenylated tryptophan in several species of fungi. They are well known from their historical role in human toxicoses. In mammals, ergot alkaloids affect the central and sympathetic nervous systems, as well as immune and reproductive systems, resulting in symptoms such as muscle contractions, changes in blood pressure, lowered immune response, reduced lactation and reproductive capability, disturbances in sleep/wake cycles, hallucinations, and gangrene of the extremities [1–4]. Different ergot alkaloids exert their effects by acting in some cases as partial agonists or, in other cases, antagonists at receptors for 5-HT (5-hydroxytryptamine or serotonin), dopamine, and noradrenaline [1,3,5]. Ergot alkaloids also affect other organisms including bacteria, nematodes, and insects [2,6–10]. Less is understood about the mechanisms behind these activities. Whereas the pharmacological effects of ergot alkaloids have been subjects of considerable research, the ecological significance of the alkaloids, which probably transcends their effects on mammals, is poorly understood.

Ergot alkaloids are produced by several fungi representing two different orders. Certain fungi in the Clavicipitaceae (order Hypocreales) produce ergot alkaloids. These include various ergot fungi in the genus *Claviceps* [1,4,11,12] and several fungi in the genera *Epichloë* and *Neotyphodium*, which live as endophytic symbionts in grasses [2,8,11]. *Aspergillus fumigatus*, a common imperfect fungus and opportunistic human pathogen with close relatives in the order Eurotiales, also produces a set of ergot alkaloids [11,13–15], many of which differ from those of the clavicipitaceous fungi. Several *Penicillium* spp., also likely derived from ascomycete ancestors in the Eurotiales, also have been reported to produce ergot alkaloids [11,14]. Not all fungi in either of these orders produce ergot alkaloids and no members of the

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lineages in between them have been reported to produce these alkaloids.

Ergot alkaloids range in complexity from simpler tricyclic alkaloids such as chanoclavine or 6,7-secolysergine to more complex tetracyclic alkaloids with tripeptide-derived side chains such as the ergopeptines (Fig. 1). They often are classified into three groups – clavines, simple amides of lysergic acid, and ergopeptines. Clavine alkaloids are the simplest ergot alkaloids and lack amide-linked side chains on the ergoline ring system. Certain clavines provide a pathway to lysergic acid, whereas others may be products of shunts off the main ergot alkaloid pathway. Still other clavines may be the ultimate pathway end product in their producing organism. Simple amides of lysergic acid and ergopeptines contain amide linkages from lysergic acid and require the activity of peptide synthetases for their formation [16–18].

Interestingly, ergot alkaloid-producing fungi typically produce a characteristic profile of several ergot alkaloids rather than a single pathway end product (Fig. 1 and Table 1). *A. fumigatus* produces a series of related clavine alkaloids, which accumulate to characteristic concentrations in or on its conidia (asexual spores), in the process of producing the ultimate pathway product fumigaclavine C. Ergot alkaloid producers in the Clavicipitaceae often have profiles that include a combination of clavines, simple amides of lysergic acid, and ergopeptines. In endophyte-infected grasses, e.g., perennial ryegrass infected with the endophyte *Neotyphodium* sp. Lp1, clavines and the ergopeptine ergovaline may be detected at relatively similar concentrations. *Claviceps africana* accumulates clavines and an ergopeptine of the dihydroergot type in its sclerotia (asexual overwintering structures, also called ergots), whereas *C. purpurea* accumulates mainly simple amides of lysergic acid and ergopeptines in its sclerotia.

The ergot alkaloid pathway appears unusually inefficient in that certain intermediates do not flow rapidly through the pathway to an ultimate end product. Instead there are typically points along the pathway at which intermediates may accumulate to concentrations approaching the same order of magnitude as the pathway end product. Also, the pathway in certain producers contains shunts along which intermediates may be diverted to alternate products. The accumulation of intermediates and alternate products (rather than their rapid conversion to the ultimate pathway product) suggests that these alkaloids provide some benefit to the producing fungus that differs from those conferred by the pathway end product.

If an inefficient pathway has been selected for because a diverse profile of alkaloids provides an advantage to the producing organism, then two predictions follow: (a) the pathway should be regulated to produce the observed profile, as opposed to being a collection of enzymes operating at randomly uncoordinated rates; and, (b) alternate end products or accumulating intermediates should have activities that differ from those of the ultimate end products. This minireview will focus on studies that address these particular points, as well as on the means by which diverse profiles of ergot alkaloids may be generated.

2. Origins of diversity in ergot alkaloid profiles

2.1. Diversification of ergot alkaloid profiles within individual producers by inefficiency

The accumulation of intermediates observed in some ergot alkaloid-producing fungi allows those intermediates to serve as de facto products as well as intermediates for the next step in the pathway. Examples of such accumulating intermediates include chanoclavine in *Neotyphodium* sp. Lp1, festuclavine and fumigaclavine A in *A. fumigatus*, and festuclavine and dihydroelymoclavine in *C. africana* (Table 1 and Fig. 1). In theory, the observed accumulation may result from differences in concentrations and/or activities of the relevant enzymes, or partitioning (by secretion or compartmentalization) of intermediates from downstream enzymes.

The hypothesis that inefficiency in the pathway is controlled rather than random is supported by studies on alkaloid accumulation in pathway knockout mutants, mRNA accumulation, and enzyme activity. Knockout of the gene encoding lysergyl peptide synthetase 1 (LPS1), controlling a late step in the pathway (Fig. 1), resulted in changes in the regulation of upstream steps in *Neotyphodium* sp. Lp1. Concentrations of clavine intermediates from the middle portion of the pathway (e.g., chanoclavine) were maintained near wild-type levels, whereas 6,7-secolysergine, believed to be produced from an early shunt in the pathway (Fig. 1), increased in concentration [18].

Coordinated regulation of genes in the ergot alkaloid pathway at the mRNA level has been demonstrated by studies on accumulation of transcripts from known and hypothesized ergot alkaloid biosynthesis genes in *C. purpurea*. Accumulation of mRNAs from genes encoding dimethylallyltryptophan (DMAT) synthase, LPS1, and LPS2, as well as seven closely linked genes in the ergot cluster (*cpox1, cpox2, cpox3, cpP450-1, cpcat2, orfA*, and *orfB*) (Fig. 2) was coordinately reduced in response to increased phosphate concentration, which represses ergot alkaloid production [16,19].

Early biochemical studies with *C. purpurea* indicated that feedback inhibition of enzyme activity also contributes to control of the pathway. Studies with semi-purified chanoclavine cyclase, which catalyzes the cyclization of chanoclavine to agroclavine, showed