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Review

SOME BIOCHEMICAL AND ECOLOGICAL ASPECTS OF THE PHYTOPATHOGENIC FUNGUS *CLAVICEPS PURPUREA*

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Abstract: *Claviceps purpurea* is a phytopathogenic fungus that grows on cereals and forage grasses. *C. purpurea* most commonly infects outcrossing species such as rye (its most common host), as well as triticale, wheat and barley. It rarely infects oats.

Three groups within this species (G1, G2 and G3) have been recognized, based on habitat association, sclerotia and conidia morphology, as well as alkaloid production. These groups have been further supported by Random Amplification of Polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers, suggesting that this species may be more accurately described as a species complex. However, all divergent ecotypes can coexist in sympatric populations with no obvious physical barriers to prevent gene-flow.

The highly poisonous purple-black sclerotia of the fungus *Claviceps purpurea* (ergot) and many other *Claviceps* species are aposematic. Highly toxic fungal sclerotia are associated with conspicuous colours (black, yellow, purple, reddish, brown, violet, white and their combinations) and severely harm herbivores that consume the infected plants, thus meeting the criteria for aposematism.

This grass-parasitic ascomycete contains numerous terpenoid indol alkaloids, some of which have dramatic physiological effects and are of great medicinal value. Ergot extracts have long been used in traditional medicine, and several isolated specific alkaloids, as well as semi-synthetic derivates of these, have proved useful remedies in modern medicine. Ergot alkaloid amide and peptide derivatives have a wide variety of physiological effects, including serotonin- and dopamine-receptor agonists and antagonists, vaso-constrictors, neurotoxins and hallucinogens.

C. purpurea produces the pharmacologically important ergopeptines, a class of cyclol-structured alkaloid peptides containing D-lysergic acid. These compounds are assembled from D-lysergic acid and three different amino acids by the non-ribosomal peptide synthetase enzymes LPS1 and LPS2. The presence of two distinct NRPS subunits catalyzing formation of ergot peptides is the first example of fungal NRPS system consisting of different NRPS subunits.

Keywords: *Claviceps purpurea*, phytopathogenic fungus, aposematism, alkaloids, physiological effects, ergopeptines, serotonin- and dopamine-receptor agonists and antagonists, vaso-constrictors, hallucinogens neurotoxins.

Introduction

Claviceps purpurea (ergot) is a biotrophic phytopathogenic fungus with a wide host range that includes four subfamilies in the grass family Poaceae: Pooideae, Arundinoideae, Chloridoideae and Panicoideae [51]. The distribution of this species is basically Holoarctic, but it has been recorded in Arctic regions [50] and also occurs in southern temperate and subtropical regions. Its centre of origin is not known, but several scientists such as Pazoutová speculate that the genus *Claviceps* had a Gondwanan origin, and most species in the genus are tropical or subtropical. It has been postulated that species close to *C. purpurea* migrated from South America to North America after the formation of the Panama land bridge, and they later spread to Europe and Africa [70].

The morphology of *C. purpurea* is variable: the sclerotial length ranges from 2 to 50 mm, and the colour of the stromata varies over a wide range of shades of red, from wine to purple

[83] and even to orange [89]. The polymorphism is also evident in conidial size and shape; the conidia range from oval spores 5 µm long to cylindrical or elongate spores up to 13 µm long [51; 83; 89].

Speciation

Species concepts for the classification of fungi are traditionally based on reproductive biology and morphology. Recently, phylogenetic approaches have become increasingly used to resolve species identification to the detriment of these concepts of taxonomic classification, which have been intensely debated among scientists. Moreover, phylogenetic analyses have challenged morphological species concepts and have been especially helpful in delineating fungal species with few morphological characters. Phylogenetic approaches have also been sought for the many fungi in which sexual reproduction is not known to occur, making biological species concepts impossible to implement. Phylogenetic studies have routinely identified cryptic species within morphological species in various fungal genera such as *Cenococcum* [17], *Coccidioides* [43], *Letharia* [44] and *Fusarium* [63].

What has not been determined in most of these studies is the mechanism behind the speciation process. In some instances, geographical isolation has been suggested, as in *Coccidioides* [43] and *Fusarium* [63], whereas in other cases these putative cryptic species may be found in the same geographical location and, in some instances, even isolated from the same soil core [80; 60; 17]. Ecological theory predicts that the stable coexistence of identical competitors will not occur [31], suggesting that cryptic species occupying the same apparent niche may play different ecological roles. Therefore, ecological factors likely play a significant role in the speciation process of co-occurring organisms. One group of fungi where ecological aspects may have influenced speciation is within the *Claviceps purpurea* complex [18].

The sclerotia of *Claviceps purpurea* contain peptide alkaloids that belong to three basic groups: the ergotamines (with alanine as the first amino acid entering the cyclopeptide moiety), the ergotoxines (with valine), and the rarely found ergoxines (with 2-amino-isobutyric acid).

For the last century, researchers have tried to use the variation in morphology and alkaloid content to establish varieties, special forms, or races [2], and the primary focus has been on detection of host-specific groups.

Stäger introduced four special forms: *secalis*, *lolii* (later united with *secalis*) [56], *milii* (on *Milium* and *Brachypodium* only), and *glyceriae* (suspected to be *Claviceps wilsonii*) for *C. purpurea* sensu Tulsane [84; 85; 86].

Stäger found that sclerotia formed on grasses from wet habitats could float on water, whereas sclerotia formed on *Secale* spp., *Lolium* spp., *Brachypodium sylvaticum*, *Sesleria coerulea*, *Arrhenatherum elatius*, *Agropyron repens* (now *Elytrigia repens*), *Alopecurus myosuroides*, and other terrestrial grasses, sank in water. On *Dactylis glomerata*, *Calamagrostis epigeios*, and some *Holcus* and *Poa* spp., sclerotia of both types were observed. For the floating isolates, the new taxon f. sp. *Phalaridis arundinaceae natans* was defined [87].

Loveless found that the conidia of isolates from grasses that originated in wet and shady habitats were longer (6.5–8.5 µm) than those from isolates found on terrestrial grasses (5–6 µm). Conidial shape and size remained unchanged, even when the isolates from different hosts were inoculated on to wheat or cultivated on agar plates [52], revealing that this trait is characteristic of an isolate and not of the substrate upon which it is raised. Another group of *C. purpurea* isolates was found on *Spartina* spp., populating Atlantic salt marshes in the Americas. This group was characterized, as analyzed by thin-layer chromatography (TLC), by the predominant production of ergocryptine, ergocryptinine and lysergylvalylmethylester [20].

Kobel and Sanglier [42] identified 10 chemoraces in sclerotia collected in Europe and North America, the most common combinations being ergocornine and ergocryptine (23% of the

samples), ergocristine and ergosine (20%), and ergotamine (13%). The composition of the alkaloid mixture produced is hereditary and independent of the host [46].

Pažoutová *et al*. studied genetic variability of *Claviceps purpurea* by using randomly amplified polymorphic DNA (RAPD), an *Eco*RI restriction site polymorphism in the 5.8S ribosomal DNA (rDNA), the alkaloids produced, and conidial morphology. They identified three groups: group G1 from fields and open meadows, group G2 from shady or wet habitats, and group G3 from *Spartina anglica* in salt marshes.

G1 isolates originated from fields, from open meadows, and from grasses growing along roads, from open, sunny localities that often are dry. It was found that *Alopecurus pratensis*, *Ammophila arenaria*, *Arrhenatherum elatius*, *Dactylis* spp., *Festuca ovina*, *Festuca rubra*, *Phleum* spp. and *Poa pratensis* could be colonized naturally by both G1 and G2 isolates.

G2 isolates were more commonly recovered from hosts growing in shady or wet habitats, and *Calamagrostis*, *Phalaroides*, *Phragmites*, and *Molinia* were the most frequent host genera. Common habitats included pond and river banks, ditches, forests, and even mountain woods [68].

G3 isolates have the longest conidia (length >10 μm); conidia shorter than 6.5 μm were always from G1 strains. Conidia between 8.5 and 10 μm long belonged to G2 strains. In the 6.5- to 8.5-μm range, G1 and G2 strains overlapped. Therefore, conidial size is only of secondary importance in distinguishing the three groups [68].

All of the sclerotia of G2 strains could float, whereas the sclerotia of G1 strains all sank within the first 30 minutes in water. The best flotation was observed with the sclerotia of G3 strains, which were difficult even to wet.

Sclerotia of G1 strains contained one or more of seven different alkaloids. Sclerotia of G2 strains contained only ergosine, ergocristine, and small amounts of ergocryptine. Sclerotia of G3 strains contained mixtures of ergocristine and ergocryptine. Thus, G2 and G3 constitute stable chemoraces [68].

In a study conducted by Douhan *et al*. on *Claviceps purpurea*, phylogenetic and population genetic analyses showed marked genetic differences among the different ecotypes and suggested little or no gene-flow among the ecotypes.

The G1 types were significantly divergent from the G2/G3 habitat types, based on each of the three loci and the combined dataset, whereas the G2/G3 types were more integrated with one another. However, although the G2 and G3 lineages have not diverged as much as the G1 lineage, based on DNA sequence data, the use of three DNA loci did reliably separate the G2 and G3 lineages. The fact that the G2 and G3 lineages are more closely related to each other than to the G1 lineage is strongly supported by the fact that sclerotia from G2 and G3 isolates float in water while those of G1 isolates sink [68; 23]. Results from his study are in agreement with previous conclusions based on AFLP and RAPD data, in which only 2% of genetic markers were shared among the three lineages of *Claviceps purpurea.* Perhaps because of their high rate of polymorphism or because of their assessment of variation across the entire genome, AFLP and RAPD data were more informative than DNA sequences for separating the different ecotypes [18].

The data from phylogenetic and population genetic analyses suggest that the three lineages G1, G2 and G3 should be recognized as unique species, or at least as varieties [18].

It is not known whether the G1, G2 and G3 groups of *C. purpurea* diverged sympatrically, or allopatrically with geographical barriers to gene-flow. Since no phylo-geographical structure was found it seems likely that ecological factors are extremely important in the speciation process of these fungi [18].

Although it remains to be determined if the three habitat-associated lineages are reproductively isolated, results from phylogenetic analysis suggest that reproduction among G2/G3 isolates would be more likely than between either G1 and G2 or G1 and G3 isolates.

However, the population genetic analyses strongly suggest little to no gene flow occurring between the different ecotypes [18]. For reproduction to occur, these different ecotypes must be able to infect the same hosts. The results of a host-range study showed that G3 isolates can infect both riparian and terrestrial grasses after artificial inoculation [69], though its range in nature is so far limited to the C4 grasses *Spartina* spp. and *Distichlis spicata*. Similarly, Pažoutová *et al*. [68; 69] reported that in the greenhouse G2 isolates can infect both riparian and terrestrial grasses, but this has not been documented under natural conditions.

Host range within *Claviceps purpurea* also suggests that ecology is more important than the host in the evolution of these fungi, which is different from many plant-associated fungi [18].

***Claviceps purpurea*: an aposematic fungus**

In chemically defended organisms, conspicuous coloration and/or patterns generally function as an advertisement of unpalatability or noxiousness to potential predators [72; 14]. The use of aposematic (or warning) signals is well documented among animal taxa [19; 76]. The effectiveness of aposematic signals is dependent on the ability of predators to form an association between conspicuous coloration and unprofitability, which results in prey avoidance [76; 56].

That mushrooms are also aposematic has been postulated; however, while some poisonous mushrooms are colourful, many edible ones are similarly colourful. For this reason, the idea of aposematic coloration in fungi [7] has not been supported as a general phenomenon by field data and taxonomic analysis [30; 79]. Taste and odour seem to be more important as common aposematic signals in large fungi, probably since many of the animals that usually consume them are nocturnal and because low levels of illumination on the forest floor would reduce the effectiveness of bright coloration as a warning signal [7, 8; 30; 79].

Lev-Yadun is one of the scientists who have brought into discussion the aposematic nature of *Claviceps* species. He asserts that the very poisonous and colourful sclerotia of *Claviceps* species are aposematic [49]. Sclerotia of *Claviceps hirtella* are yellow, those of *C. glabra* brown, those of *C. viridis* green, and those of *C. purpurea* dark purple or black – typical colours of poisonous aposematic organisms.

The association of unpalatable, highly toxic fungal organs (sclerotia) with conspicuous colours (black, yellow, purple, reddish, brown, violet, white, and their combinations), and the well documented toxicity to herbivores that consume them, meet the criteria for characterizing the association between *Claviceps* and grasses as operative aposematism [49].

Ergot infests cereals that grow in open habitats where grazers usually feed during daytime, phenomenon which offers optimal conditions for visual aposematism.

The plant/*Claviceps* interactions may be viewed simply as a disease, or as a mutualistic relationship. Defensive mutualism between plants and fungi is well known [10; 6; 64; 12], but has not been discussed as an aposematic effect. Fungal endophytes in the genus *Neotyphodium* (Ascomycetes: Clavicipitaceae) form mutualistic associations with a variety of grasses [11; 4]. The fungal hyphae grow inter-cellularly in leaf and stem tissues, causing infections that are transmitted exclusively through the seeds of the host plant. The fungus benefits from access to plant nutrient and photosynthetic resources, while the plant benefits from enhanced resistance to insect herbivores or vertebrate grazers [11; 4; 21]. A series of fungal endophyte-mediated alkaloids provides the basis for the acquired chemical defence against herbivory [71; 35]. Ergot fungi parasitize rye and other grasses, reducing their reproduction, the very poisonous fungus harming the herbivores that eat the infected plants. Potentially, this benefits individual plants, and probably nearby plants too.

Since *Claviceps* species need plant hosts, the more toxic the ergots are to herbivores, the better for both ergot and host. Chemicals in the sclerotia may also directly prevent their consumption by herbivores or from attacks by other fungi and micro-organisms. Indeed, large herbivores usually learn to avoid toxic plants [34; 48], resulting in a reduced tendency to

consume infected hosts. Alternatively, ergot fungi may induce abortions or kill the animals that eat ergot-infested species, decreasing grazing pressure. Since low levels of ergot consumption are not lethal, but cause sickness [10; 58], conditions are appropriate for the development of food aversion towards ergot-infected grasses. The array of alkaloids in ergots makes it difficult for herbivores to evolve resistance to ergot toxicity. When ergot fungi protect their host populations against herbivory, they protect their own habitat and benefit the host plants. They also reduce the host's evolutionary tendency to enhance its resistance to the disease. The association between *Claviceps* and grasses appears to be a fine-tuned ecological tactic that fits the definition of "dangerous liaisons" *sensu* van Baalen and Jansen (2001). Odour, in addition to colour, might be involved in the aposematic signalling of ergots even in their open, well-illuminated habitats as was found for fungi that grow in dark habitats, as described by Camazine [7; 8], Guevara & Dirzo [30] and Sherratt *et al*. [79].

Ergot alkaloids are fungal metabolites that have a long history as mycotoxins. While they have damaging effects on the central nervous system (CNS), they also enjoy a long biotechnological tradition, with manifold applications in the therapy of human CNS disorders. Chemically, the ergot alkaloids are 3,4-substituted indol derivatives having a tetracyclic ergoline ring structure.

Biosynthesis of ergot alkaloids; the pathway of ergoline ring formation

The main steps of the pathway of ergoline ring formation were primarily established by *in vivo* precursor studies in alkaloid-forming *Claviceps purpurea, C. fusiformis*, or *C. paspali* [25; 26; 28; 38]. By contrast, the enzymology of ergoline ring formation has been a slowly progressing field apparently because of the instability of most of the enzymes involved in cell-free extracts from *Claviceps* spp. The only exception to this is dimethylallyltryptophan (DMAT) synthase, which catalyzes the condensation of L-tryptophan with dimethylallylpyrophosphate [32]. Only limited information is available for a few other enzyme activities involved in the ergoline pathway; these were investigated in partially purified protein fractions. DMAT synthase, which produces dimethylallyltryptophan, has been purified to homogeneity and found to be an α_2 dimer of 105 kDa [27; 47]. The DMAT-forming reaction is the committed step of alkaloid synthesis in ergot fungi and delivers the carbon skeleton of the ergoline ring system. Moreover, expression of the enzyme is genetically controlled by tryptophan, which induces alkaloid synthesis and relieves repression by phosphate, a most important issue in the regulation of ergot alkaloid biosynthesis [45; 75]. The DMAT synthase gene has been cloned from both *Claviceps fusiformis* and *Claviceps purpurea* [90; 91]. The *C. fusiformis* gene was expressed in *Saccharomyces cerevisiae* and shown to produce a catalytically active enzyme. Interestingly, the DMAT synthesase gene displayed almost no similarity to other prenyltransferase sequences, with the exception of a possible prenyl diphosphate moiety (DDSYN) at position 113–117 of the amino acid sequence. This moiety is also conserved in other farnesyl diphosphate and geranylgeranyl diphosphate synthases [82]. All of the enzymatic steps after DMAT formation concern modifications and rearrangements leading to formation of rings C and D. *N*-methylation of the amino nitrogen of DMAT is catalyzed by a methyltransferase, which was analyzed with respect to its kinetic constants and substrate specificity [65; 66]. The methylation step is followed by decarboxylation and closure of ring C, a reaction in which chanoclavine-I cyclase is involved [22; 29]. Except for its substrate requirements, chanoclavine-I-cyclase was not further characterized and it is not clear which particular step of the mechanism of chanoclavine-I cyclization is catalyzed.

Another known enzyme activity of the pathway is that of agroclavine-17-monooxygenase [39], which exclusively converts agroclavine to elymoclavine. Elymoclavine in turn is transformed into paspalic acid by elymoclavine-17-monooxygenase [40; 54]. Both oxygenases are dependent on NADPH and molecular oxygen and therefore most probably are cytochrome

P450- monooxygenases. Interestingly, elymoclavine-17-monooxygenase was not detected in a *Claviceps* strain that produces as end products agroclavine and elymoclavine. By contrast, the enzyme was present in a strain that produces D-lysergic acid amides and peptides [40; 54]. This may indicate that the clavine- producing *Claviceps* strain lacks the enzyme that converts elymoclavine to paspalic acid [92]. Paspalic acid itself is spontaneously isomerized to D-lysergic acid. It is most noteworthy that the pathway of ergot alkaloid formation may end at different stages, such as at chanoclavine-I, a representative of the so-called bisseco ergolenes, formed as the main compound in a number of strains of *C. fusiformis*. In other *C. fusiformis* strains the pathway ends at the stage of agroclavine or elymoclavine, the two main representatives of the clavinet group of alkaloids. In strains of *C. paspali* or *C. purpurea*, the alkaloid pathway continues through elymoclavine to the next intermediate, paspalic acid, the immediate precursor of D-lysergic acid from which the next step leads to the simple D-lysergic acid amides or to the ergopeptines, the classical ergot peptide alkaloids of *C. purpurea* [24]. The different variants of the alkaloid biosynthesis pathway found in nature may be the result of natural mutations in the downstream genes of the clavine assembly genes or a complete lack of the relevant genes.

Assembly of ergot peptides and simple D-lysergic acid amides

A major issue of ergot alkaloid biosynthesis research is the investigation of the formation of the various amide derivatives of D-lysergic acid. To these belong the ergopeptines, in which D-lysergic acid is attached to the bicyclic cyclol-lactam structure formed from three amino acids [33]. The numerous ergopeptines differ from each other by amino acid substitutions in the first two amino acid positions (i.e. the ones adjacent to D-lysergic acid) of the cyclol-lactam chain while the third (containing proline) is invariable. The variable amino-acids are aliphatic ones with linear and branched side chains (alanine, valine, isoleucine, leucine, aminobutyric acid) and the amino acid phenylalanine. Ergopeptines found in nature are classified into the ergotamine, ergoxine, and ergotoxine groups depending on their amino acid composition [91].

The ergopeptines are derived from D-lysergyltripeptide lactam precursors. The latter are enzymatically converted to the corresponding ergopeptines by a cytochrome-p450-related activity, which hydroxylates the first amino acid of the peptide chain [73]. The D-lysergyltripeptide lactams are assembled from D-lysergic acid and the amino acids of the peptide lactam chain by a non-ribosomal mechanism [37]. The enzyme D-lysergyl peptide synthetase (LPS) has been purified from crude extracts of *C. purpurea* [74]. It is a typical non-ribosomal peptide synthetase (NRPS) which activates D-lysergic acid and the three amino acids of the peptide portion of the alkaloid as adenylates and binds them as thioesters [57]. The enzyme consists of four modules, each housing domains for adenylation, thiolation, and condensation of the constituents of the ergot peptide alkaloid backbone. Interestingly, LPS consists of two large subunits, which is unusual because, as yet, all described fungal NRPSs consist of a single polypeptide chain. The larger (370 kDa) polypeptide (LPS1) activates the amino acids of the peptide portion. The smaller (140 kDa) subunit is responsible for D-lysergic acid activation (LPS2) [92]. The analysis of enzyme-bound reaction intermediates accumulating on LPS in the course of D-lysergylpeptide lactam synthesis revealed that peptide synthesis starts when D-lysergic acid binds to the LPS2 subunit. By condensation of D-lysergic acid with the amino acid bound to the first module of LPS1, a D-lysergyl- monopeptide is formed as the first intermediate and sits in the first module of LPS1. LPS1 then catalyzes the successive condensation of the D-lysergylmonopeptide with the next two amino acids bound to modules 2 and 3 of LPS1. Finally, the D-lysergyltripeptide is released from LPS1 by cyclization to the lactam [74; 94].

In contrast to the D-lysergyl-tripeptides, the mechanism of formation of the simple D-lysergic acid amides D-lysergic acid α-hydroxyethylamide and ergometrine is unknown. Precursor studies indicate that they are formed from D-lysergyl-alanine [1; 9]. This indicates a specific D-lysergyl peptide synthetase operating in the case of the simple D-lysergic acid amides.

This NRPS may contain two modules, one responsible for D-lysergic acid activation and the other for alanine activation. As yet, neither this enzyme nor the reaction leading from D-lysergylalanine to ergometrine or d-lysergic acid α-hydroxyethylamide is known [38].

Ergot alkaloid pharmacodynamics

The pharmacological effects of the ergot alkaloids as a group tend to be complex and variable, the net result of their actions being a sum of the effects of partial agonism or antagonism at adrenergic, dopaminergic, and serotonergic receptors. Variables relating to these effects are influenced by the agent, dosage, species, tissue, physiological and endocrinological state, and experimental conditions.

Ergonovine (Ergometrine, Ergobasine)

Ergonovine was discovered almost simultaneously in four different laboratories, with four different names (ergometrine, ergotocine, ergosterine, and ergobasine) assigned to this alkaloid.

The structure of ergonovine was elucidated in 1935 when it was shown that hydrolysis of the alkaloid afforded (1)-lysergic acid and (1)-2-aminopropanol. Ergonovine is a light-sensitive, water-soluble compound that is commercially marketed as its water-soluble maleate salt. The compound is currently obtained from three different sources: isolation from field ergot as a minor by-product, isolation from fermentation broth, and synthesis from (1)-lysergic acid and L-(1)-2-aminopropanol using variable coupling reagents.

Ergonovine is a selective and moderately potent tryptaminergic receptor antagonist in various smooth muscles, being only a partially agonistic or antagonistic at tryptaminergic receptors in the central nervous system. In blood vessels the alkaloid is only weakly antagonistic of dopaminergic receptors and partially antagonistic of a-adrenergic receptors. The most pronounced effect of ergonovine is one of direct stimulation of uterine smooth musculature, resulting in increased muscle tone and an enhancement of the rate and strength of rhythmical contractions. This stimulant effect seems to be most closely associated with agonist or partial agonist effects at 5-HT2 receptors.

Ergonovine maleate is typically administered intramuscularly or intravenously, with the intravenous route reserved for emergency use. The drug is contra-indicated for use in the induction of labour because it may jeopardize placental blood flow and foetal oxygen supply, and in cases of threatened spontaneous abortion, or in pregnancy. Adverse reactions are generally gastro-intestinal and are limited to nausea and vomiting (1–10%). Ergonovine derivatives are substrates of CYP3A4 metabolism, and as such are contra-indicated for concomitant use with compounds established as strong CYP3A4 inhibitors (including protease inhibitors, some macrolide antibiotics, quinolones, azole antifungals) because of the production of acute ergot toxicity. Finally, the drug has an off-label indication for use as a diagnostic test for Prinzmetal's angina (variant angina, vasospastic angina) in which it has been used successfully for non-invasive diagnosis of coronary vasospasm as a cause of chest pain [77; 88; 15; 36].

Methylergonovine

Methylergonovine does not occur naturally in ergot, but was first introduced as a synthetic product for medical use in 1946. The drug is currently prepared via the reaction of (1)-lysergic acid with L-(1)-aminobutanol, using different coupling reagents. Unlike its homologue ergonovine, methylergonovine has low water solubility and as such is marketed as its water-soluble maleate salt. It is used therapeutically much in the same manner as ergonovine [15].

Methysergide

Methysergide is another alkaloid-derivative that does not occur naturally in ergot, but was first introduced into medical use as a synthetic product in 1960. The drug is currently prepared via synthesis from (1)-lysergic acid or by methylation of ergonovine (ergometrine, ergobasine) and is marketed as its water-soluble maleate salt. This semi-synthetic compound is a potent 5-HT2 receptor antagonist that is assumed to stabilize neurotransmission in the trigeminovascular system to block the development of neurogenic inflammation. The compound is known to produce retroperitoneal, pleuorpulmonary, and endocardial fibrosis on long-term administration in a small number of patients (0.02%). Gastro-intestinal upset is a common adverse effect [15; 41].

Ergotamine

Ergotamine is a partial agonist at various tryptaminergic receptors (including the serotonin receptor [5-HT2]) and at various *a*-adrenergic receptors in blood vessels and various smooth muscles. It is likely that the major activity of ergotamine and related alkaloids is one of agonism at the 5-HT1B/1D receptors, just as with the 'triptan' anti-migraine compounds. FDA-labelled indications for ergotamine tartrate are in the abortion or prevention of vascular headaches, such as migraine, migraine variant, cluster headache, and histaminic cephalalgia. The alkaloid is considered useful in the therapy of moderate to severe migraine attacks, in which it acts to constrict intracranial blood vessels and inhibit the development of neurogenic inflammation in the trigemino-vascular system. Both venous and arterial constriction occur at therapeutic dosage. Ergotamine is most effective when administered early in the migraine attack, preferably at the first indication of an impending event. Nausea and vomiting are the most common adverse effects of therapy due to stimulation of the medullary chemoreceptor trigger zone. Other common adverse effects include muscle weakness, fatigue, paresthesias, tightness in the chest, and diarrhoea. Prolonged administration or excessive dosage may result in severe peripheral vasoconstriction, ergotism, gangrene, or various fibrotic complications (cardiac valvular, retroperitoneal, pleuro-pulmonary). Ergotamine derivatives are contra-indicated in patients with peripheral vascular disease, hepatic or renal disease, coronary artery disease, hypertension, sepsis, or pregnancy.

Dihydroergotamine

Dihydroergotamine, which does not occur naturally in ergot, was first introduced as a semi-synthetic product in 1946. The drug is currently prepared either by hydrogenation of ergotamine isolated from field ergot/fermentation broth or via synthesis from dihydrolysergic acid and the appropriate synthetic tripeptide. Dihydroergotamine has very low water solubility and is marketed for parenteral use as its water-soluble mesylate (methanesulfonate) salt. FDA-labelled indications for the use of dihydroergotamine mesylate are for the acute treatment of the symptoms of migraine headache and for the acute treatment of cluster headache. The compound is poorly and variably absorbed from the gastro-intestinal tract and for this reason is available for intranasal and parenteral (intravenous, intramuscular, subcutaneous) administration. Following intranasal administration, the relative bioavailability is 30–40%, with peak plasma concentrations reached in 30–60 minutes. Although dihydroergotamine is rarely associated with serious toxicities, the adverse effect profile resembles that of ergotamine. Contraindications, precautions, and drug interactions are similar to those of ergotamine. Although parenteral administration was once mainly regarded as therapy reserved for emergency department treatment for moderate to severe migraine, patients have been trained to administer the drug intramuscularly or subcutaneously. The administration of dihydroergotamine mesylate is not generally associated with the production of rebound headache, but dosage restrictions cited for ergotamine tartrate should be strictly observed to prevent this phenomenon [15; 53].

Bromocriptine

Bromocriptine (2-bromoa- ergocriptine) is not a naturally occurring ergot alkaloid, but is a semisynthetic derivative of the naturally occurring peptide alkaloid a-ergocriptine (a-ergocryptine, a-ergokriptine, a-ergokryptine) that has been isolated from field ergot/fermentation broth or prepared via synthesis from (1)-lysergic acid. Bromocriptine is prepared via the bromination of a-ergocriptine with different brominating agents and is marketed as its water soluble mesylate (methanesulfonate) salt. Although bromocriptine exhibits only weakly antagonistic interactions with tryptaminergic and a-adrenergic receptors, the drug is a potent dopamine receptor agonist via activation of certain central dopamine D2 receptors. Bromocriptine is known to stimulate both pre- and postsynaptic sites, promoting the release of dopamine and inhibiting dopamine uptake. The net effect is a decrease in the turnover rate of dopamine without significant changes in concentration. Bromocriptine inhibits the release of prolactin by direct stimulation of postsynaptic dopamine receptors in the hypothalamus. Indications for bromocriptine are for the therapy of hyperprolactinemia and pituitary prolactinoma, in the therapy of acromegaly and as an adjunct to L-dopa therapy in patients with Parkinson's disease who are experiencing a deteriorating response to L-dopa or who are undergoing fluctuations in response to the drug. Other patients who may benefit from bromocriptine therapy include those with limited clinical response to L-dopa due to an inability to tolerate higher doses. Dopamine agonist drug therapy is associated with an L-dopa-sparing effect and with a decrease in the frequency of periods. Adverse effects of therapy may be dose-limiting, tending to occur more frequently at the beginning of therapy, and being more likely with higher dosage or rapid escalation of dosage.

Gastrointestinal upset (particularly nausea) is common and may also be accompanied by asymptomatic postural hypotension, sedation, light-headedness, and vivid dreams. Daytime sedation, confusion, and hallucinations are psychic effects that are often dose limiting [77; 61; 78; 15].

Lysergic acid diethylamide

Lysergic acid diethylamide (German: *lysergsauerediethylamid*) was first synthesized in 1938 in a screening of compounds for oxytocic activity and was the 25th semi-synthetic ergot that Dr Albert Hofmann of Sandoz AG in Basel, Switzerland, prepared by combining (1)-lysergic acid with different amines. The compound was tested in comparison with ergonovine and found to have less oxytocic activity. LSD and related hallucinogens are known to interact with brain 5-HT receptors to produce agonist or partial antagonist effects on serotonin activity. This includes both the presynaptic 5-HT1A and 5-HT1B receptors, as well as the post-synaptic 5-HT2 receptors. Theoretically, LSD and other hallucinogens promote glutamate release in thalamocortical terminals, thus producing dissociation between sensory relay centres and cortical output. It is not known whether the agonist property of these hallucinogens at 5-HT2C receptors contributes to behavioural alterations. LSD has also been demonstrated to interact with many other 5-HT receptors, including cloned receptors of which the functions have not yet been determined. In any event, the precise mechanism by which hallucinogens exert their effects remains unsolved. The onset of the action of LSD is within 30–60 minutes, with effects peaking over 1–6 hours and dissipating in 8–12 hours. Acute toxicity includes gastro-intestinal upset, chills, hyperglycemia, hypertension, mydriasis, tachycardia and panic, while chronic effects include flashbacks, and exacerbation of latent mental disorders, particularly schizophrenia. Because of its relatively high therapeutic index, no deaths have been directly attributed to LSD use alone [81; 59; 16; 62; 13].

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ASPECTE BIOCHIMICE ŞI ECOLOGICE ALE CIUPERCII FITOPATOGENE *CLAVICEPS PURPUREA*

(Rezumat)

Claviceps purpurea este o ciupercă fitopatogenă care se dezvoltă pe cereale şi plante furajere. *C. purpurea* infectează în special secara (gazda sa cea mai comună), precum şi triticale, grâu şi orz. Ea afectează doar rar ovăzul. În interiorul acestei specii au fost recunoscute trei grupuri (G1, G2 şi G3) pe baza asocierii habitatelor, morfologia conidiilor şi scleroţilor, precum şi a producţiei de alcaloizi. Existenţa acestor trei grupuri a fost susţinută şi de către Amplificarea Randomică a ADN-ului Polimorfic (RAPD), precum şi de către markeri ai analizei polimorfismului de lungime a fragmentelor de ADN amplificate (AFLP), sugerând că această specie ar putea fi descrisă mai corect ca un complex de specii. În orice caz, toate ecotipurile divergente pot coexista în populaţii simpatrice fără existenţa unei bariere fizice evidente care să prevină fluxul de gene.

Sclerotul foarte otrăvitor de culoare mov-negru al ciupercii *Claviceps purpurea* (ergot) precum şi alte specii de *Claviceps* sunt aposematice. Scleroţii foarte toxici sunt asociaţi cu colorаţii evidente (negru, galben, mov, roşiatic, maro, violet, alb, precum şi combinaţiile lor) şi produc daune severe erbivorelor care consumă plantele infectate, întrunind astfel criteriile pentru aposematism.

Ascomyceta parazită conţine numeroşi alcaloizi indol terpenoidici, printre care unii au efecte fiziologice puternice şi o foarte mare valoare medicinală. Extractele din scleroţi au fost utilizate în medicina tradiţională timp îndelungat, iar câţiva izolaţi alcaloidici specifici, precum şi derivaţi semisintetici s-au dovedit a fi remedii folositoare în medicina modernă. Alcaloizii amidici şi derivaţii peptidici din scleroţi prezintă o gamă largă de efecte fiziologice, inclusiv agoniste şi antagoniste receptorilor dopaminici şi serotoninici , vasoconstrictoare, neurotoxice şi halucinogene.

C. purpurea produce ergopeptine cu importanţă farmaceutică; ele sunt o clasă de alcaloizi de origine peptidică cu structură ciclolică ce conţine acidul D-lisergic. Aceşti compuşi sunt asamblaţi din acidul D-lisergic şi trei aminoacizi diferiţi de către enzimele peptid-sintetaze nonribozomale LPS1 şi LPS2. Prezenţa a două subunităţi NRPS distincte ce catalizează formarea peptidelor sclerotice este primul exemplu de sistem NRPS compus din două subunităţi diferite întâlnit la fungi.

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