Fungal Hallucinogens Psilocin, Ibotenic Acid, and Muscimol: Analytical Methods and Biologic Activities

Katarzyna Stebelska, PhD

Abstract: Psychoactive drugs of fungal origin, psilocin, ibotenic acid, and muscimol among them have been proposed for recreational use and popularized since the 1960s, XX century. Despite their well-documented neurotoxicity, they reached reputation of being safe and nonaddictive. Scientific efforts to find any medical application for these hallucinogens in psychiatry, psychotherapy, and even for religious rituals support are highly controversial. Even if they show any healing potential, their usage in psychotherapy is in some cases inadequate and may additionally harm seriously suffering patients. Hallucinogens are thought to reduce cognitive functions. However, in case of indolealkylamines, such as psilocin, some recent findings suggest their ability to improve perception and mental skills, what would motivate the consumption of “magic mushrooms.” The present article offers an opportunity to find out what are the main symptoms of intoxication with mushrooms containing psilocybin/psilocin, muscimol, and ibotenic acid. The progress in analytical methods for detection of them in fungal material, food, and body fluids is reviewed. Findings on the mechanisms of their biologic activity are summarized. Additionally, therapeutic potential of these fungal psychoactive compounds and health risk associated with their abuse are discussed.

Key Words: hallucinogens, psilocin, ibotenic acid, muscimol

INTRODUCTION
Psychoactive plants or fungi accompanied religious rituals in many ancient civilizations worldwide. Hallucinogenic compounds contained in fungi of Psilocybe and Amanita genera have been used as recreational drugs of abuse since the beginning of the 1960s, XX century. Although hallucinogens are thought to be safe because of relatively low physiologic toxicity and dependence potential nowadays the context of hallucinogenic compounds consumption, mainly by young people seeking for unusual experiences, is usually negative but not exclusively. One exception seems to be the idea to use them for religious ceremonies or psychotherapy support. As an example, it was experimentally established that psilocybin, psilocin prodrug of fungal origin, is able to produce mystical states under favorable circumstances (involving acquired cultural frame of references). Some details of the study design, particularly the usage of niacin as an active placebo, may suggest that the authors of the first of these experiments known as Good Friday Experiment were aware that religious rituals alone are not neutral for mental health. The states of altered consciousness induced by religious settings and facilitated by psychedelic drugs increase suggestibility to suggested messages and are not free from negative emotions such as fear, terror, anxiety, shame, and sense of guilt. Therefore, any religious cult, particularly if combined with psychoactive compounds intake, should be considered as being able to alter psychosocial identity. Psychotic syndromes evoked in religious purposes are not fully safe and may induce development of really serious psychopathologies including confusional syndrome and schizophrenia, a disease of at least partly psychosocial etiology. It should be pointed out that psilocybin/psilocin, although still scientifically explored as potentially helpful and safe psychotherapy adjunctive, is presently used to model schizophrenia and to investigate psychosis similar to positive symptoms of the disease.

The psychoactive principles of Psilocybe mushrooms, namely psilocybin and psilocin, were isolated and identified by Hofmann in 1958. Both these substances, similarly as LSD, were originally proposed as incapacitating agents and used for intelligence purposes. Ibogenic acid and muscimol seemed to be the most active substances of fly agaric responsible for its hallucinogenic properties. Exposure to these substances, especially chronic, not only may constitute a significant risk to human mental health but may also induce other serious disorders including cardiovascular complications and neurodegenerative diseases. Although chemically unstable, ibogenic acid should be considered as highly toxic agent if one is aware of convulsant activity and excitotoxicity of its endogenous analogue glutamate. Simultaneously, the fungal psychedelics of high biologic activity display significant therapeutic potential.

Hallucinogenic fungi and psychedelics isolated from them are illegal in many countries or their processing and distribution stay under strict regulation. However, because they are easily available from natural habitats, they have become quite popular among growing population of drug users and are frequent cause of serious poisoning or even accidental deaths resulted from their biologic activity.
Therefore, analytical methods elaborated for detection of these substances in fungal material and body fluids are also reviewed in this article.

Psilocybin/Psilocin as a Secondary Metabolite

Psilocybin is a secondary metabolite of some species of Agrocybe, Conocybe, Copelandia, Galerina, Gymnopolis, Hypholoma, Inocybe, Panaeolina, Panaeolus, Pholiota, Pluteus, and *Psilocybe* genera.  The major part of the known psychoactive species belongs to the genus *Psilocybe*. They are saprotrophic on wood, dung, moss, soil, and litter. *Psilocybe cyanescens*, *P. serbica* var. *bohemica*, *P. serbica* var. *arcana*, and *P. semilanceata* are probably the only species containing sufficient for intoxication amounts of psilocin/psilocybin that can grow in natural or near natural habitats in the Middle and North Europe. *Psilocybe cubensis* is the most popular natural source of psilocybin in the warmer regions of the Americas. In the United States, it can be found in the wild throughout the southeast to Texas and the Pacific Northwest. The fungus is common in Mexico and other Central American countries, and it is also known from South American, Asian, Australian and Oceanian localities. *Psilocybe cubensis* is widespread in New Zealand and Tasmania.

Season-dependent availability of psilocybin-containing mushrooms has stimulated the search for methods to cultivate these fungi. Culturing of the mushrooms became common after reports on the methodology seemed in both scientific literature and specialist books. It seems that most recreationally used mushrooms are cultivated rather than picked wild at present. For illicit home growers, *P. cubensis* and its varieties are generally the mushrooms of choice because they are easy to culture and fruit readily in vitro, producing a significant amount of biomass with each flush.

Alkaloids isolated from hallucinogenic mushrooms within *Psilocybe* genus such as psilocybin, baeocystin, nor-baeocystin, and the tryptophan derivatives formed through decarboxylation, indole ring hydroxylation at position 4, and N-methylation through the activity of S-adenosylmethionine and O-phosphorylation. Biosynthetic pathway of psilocybin in *P. cubensis* involves conversion of tryptophan to tryptamine, N-methyltryptamine, N,N-dimethyltryptamine, and psilocin or alternatively conversion of 4-hydroxytryptamine to psilocybin. *Psilocybe cubensis* cultures have the ability to hydroxylate tryptamine derivatives at position 4.

*Psilocybe* spores and monocaryotic mycelium do not contain psychoactive compounds. Mycelium starts to produce psilocybin/psilocin at further stages of mushroom development. Quantitative analysis of *P. cubensis* fruit bodies collected from natural sources or from artificial cultures grown under strictly controlled conditions reveals that the content of psilocin varies greatly (eg, from a 3.2–13.3 mg/g for artificial culture). The first flush collected from culture cultivated on rice grain generally does not contain psilocin.

The amount of psilocybin depends on composition of nutrient medium. Media depleted in source of carbon are less effective in the alkaloid production. Tryptophan supplementation stimulates psilocybin biosynthesis. This happens when tryptophan is present in the medium from the stage of culture inoculation. Replacement of nutrient medium does not affect psilocybin content. Conditions of potassium depletion do not influence considerably mycelium growth but lower psilocybin production. Lack of succinate in the medium decreases biosynthesis of psilocybin.

Psilocybin seems to be present at higher amounts in mushroom caps than in the stems. *Psilocybe semilanceata* and *P. serbica* var. *bohemica* (synonymous with *P. bohemica*) were found to contain high amounts of psilocybin up to 1% of dry weight. *Psilocybe cubensis* seemed to contain minor amounts of psilocybin and low amounts of psilocin (0.63% and 0.11%, respectively). The contents of psilocybin and psilocin in the sample of *P. cyanescens* were 0.32% and 0.51%, respectively. *Psilocybe serbica* var. *bohemica* collected in Europe were found to contain minor amounts of psilocybin (content between 0.33% and 0.62%) and almost no psilocin. *Psilocybe cyanescens* from the Pacific Northwest, United States, contained both psilocybin (0.41%–0.98%) and psilocin (0.23%–0.93%).

During mycelium development, some processes are controlled by light or are light responsive, and psilocybin biosynthesis is also somehow under control of ultraviolet (UV) radiation. *Psilocybe* genus mushrooms produce fruit bodies after UV or blue light irradiation. The caps of fruit bodies formed from poorly enlightened cultures develop bluish phototropic zone originating from oxidation products of indole compounds. It is also thought that the highest level of psilocybin coincides with sporulation time.

Analytical Methods Used for Psilocybin/Psilocin Detection and Isolation

Methods of Extraction

Psilocybin due to the presence of highly polar phosphate group is well soluble in water but poorly soluble in methanol and ethanol. Psilocybin is practically insoluble in nonpolar solvents such as chloroform and benzene. Psilocin is slightly more soluble in some nonpolar solvents (1-chlorobutane or dichloromethane). Water recrystallized psilocybin (melting point of 220–280°C) forms white needles and contains crystal water. Psilocybin recrystallized from methanol contains crystal methanol and melts at 185–195°C.

Preparation of fungal material to extraction usually consists in its air drying at room temperature or slightly elevated temperature (but not above 40°C) and/or freeze-drying. Comparable analysis reveals that freeze-dried material contains the highest amounts of psilocybin/psilocin. Pulverization of dried material is obtained by the use of a mortar or in laboratory mills and homogenizers. Macerated homogenates prepared in a mortar demand separation of solid components.

Extraction of dried and powdered material is usually assisted by mechanical agitation. Sonication in a water bath was used to assist extraction process. To obtain the extract of psilocybin/psilocin, a micropercolator was also used. The time of extraction, depending on the amount of material, solvent volume, and the exact mechanical agitation technique, ranges from a few minutes to several hours.

Analytical Methods Used for Psilocybin/Psilocin Detection and Isolation

Methods of Extraction

Psilocybin due to the presence of highly polar phosphate group is well soluble in water but poorly soluble in methanol and ethanol. Psilocybin is practically insoluble in nonpolar solvents such as chloroform and benzene. Psilocin is slightly more soluble in some nonpolar solvents (1-chlorobutane or dichloromethane). Water recrystallized psilocybin (melting point of 220–280°C) forms white needles and contains crystal water. Psilocybin recrystallized from methanol contains crystal methanol and melts at 185–195°C.

Preparation of fungal material to extraction usually consists in its air drying at room temperature or slightly elevated temperature (but not above 40°C) and/or freeze-drying. Comparable analysis reveals that freeze-dried material contains the highest amounts of psilocybin/psilocin. Pulverization of dried material is obtained by the use of a mortar or in laboratory mills and homogenizers. Macerated homogenates prepared in a mortar demand separation of solid components.

Extraction of dried and powdered material is usually assisted by mechanical agitation. Sonication in a water bath was used to assist extraction process. To obtain the extract of psilocybin/psilocin, a micropercolator was also used. The time of extraction, depending on the amount of material, solvent volume, and the exact mechanical agitation technique, ranges from a few minutes to several hours.

© 2013 Lippincott Williams & Wilkins
of extraction improves and its time may be shortened if the procedure is carried out at elevated temperature.\textsuperscript{96} However, heating to the temperature above 70°C leads to psilocybin dephosphorylation, particularly if the extraction is carried out at acidic pH conditions.\textsuperscript{82,91} To improve effectiveness, extraction may be repeated several times and the obtained extracts combined before analysis.\textsuperscript{89,90,97,98} Methanol is the most often used solvent for extraction of psilocybin/psilocin because it is able to denature proteins and to prevent enzymatic conversion of psilocybin to psilocin.\textsuperscript{55,75,78,87,89,90,92,97} According to Kysilka and Wurst,\textsuperscript{94} the optimal solvent for the extraction of psilocybin is acetate which is also sometimes used for extraction.\textsuperscript{89} A procedure of 1-step extraction directly to chloroform was also described.\textsuperscript{95} Sometimes a method suitable for alkaloids isolation is used, namely the extraction to acidified water solution followed by extraction to nonpolar solvent at basic pH.\textsuperscript{78,82,83,91,99,100} The macerated material is heated during the procedure what leads to conversion of psilocybin to psilocin.

Several examples of extraction methods used for psilocybin/psilocin analysis are briefly described in Table 1.

To analyze body fluids (plasma or urine) for the presence of psilocin, it is necessary to hydrolyze conjugate of psilocin with glucuronic acid. It is usually performed by the use of \textit{Escherichia coli} or Helix pomatia β-glucuronidase at slightly acidic conditions (pH 5–6).\textsuperscript{102–105} Acidic or alkaline hydrolysis was reported to be ineffective.\textsuperscript{104} The addition of methanol to the sample denatures proteins.\textsuperscript{104} To deproteinize the ice cold sample, 20% of the solution of polyethylene glycol 6000 was also used.\textsuperscript{84} Centrifugation is an effective method to separate precipitated proteins from supernatant containing the exact analytes.\textsuperscript{104}

The obtained supernatant after evaporation to dryness and dissolving in appropriate solvent is usually directly analyzed without further treatment. Sometimes the samples undergo solid-phase extraction for initial purification. For example, Chem Elut column was used to extract body fluid samples.\textsuperscript{55} Initial purification of urine samples containing psilocin was performed by means of solid-phase extraction using cation-exchange sorbent.\textsuperscript{105} To isolate indole alkaloids from fungal tissue, the crude methanolic extract was purified by ion-exchange chromatography using cation-exchange sorbent.\textsuperscript{79} Purification and preconcentration could be performed using cartridges containing octadecyl sorbent.\textsuperscript{106} Before high-performance liquid chromatography (HPLC) analysis, psilocin-containing samples after deproteinization and alkalization to pH 8.5 were extracted using liquid–liquid extraction technique or online solid-phase extraction with cation-exchange sorbent.\textsuperscript{84}

### Methods of Qualitative Analysis and Isolation

Blue compound being a product of psilocin oxidation is easily formed in iodine vapors. Enzymatic oxidation of psilocin is catalyzed by p-diphenol oxidase.\textsuperscript{107} Similarly, oxidation with ferric chloride, a component of the Keller reagent used for Thin Layer Chromatography (TLC) detection of indole compounds, makes possible visualization of psilocin spots on TLC plates.\textsuperscript{51,60}

Under UV illumination, psilocin is visible as absorbent spot.\textsuperscript{108} Psilocybin shows blue fluorescence under UV light.\textsuperscript{108} Identification of indole compounds such as psilocybin and psilocin in water solutions or after chromatographic separation on TLC plates may be performed applying the Ehrlich reagent containing p-dimethylbenzaldehyde or para-Dimethylcinnamic acid, 2,4-dimethylcinnamic acid reagent containing 4-(dimethylamino) cinnamaldehyde.\textsuperscript{73,75,108–110} Psilocin was visualized with diazotized p-nitroaniline followed by alkali.\textsuperscript{105} Psilocin identification may be based on UV and infrared spectrometry.\textsuperscript{83}

Thin-layer chromatography was reported as an appropriate method of psilocybin/psilocin isolation using cellulose

---

**TABLE 1. Exemplary Methods of Extraction Used for Psilocybin/Psilocin Determination**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Extraction Time</th>
<th>Dry Material/Solvent Ratio</th>
<th>Extraction Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>12 h</td>
<td>250 mg/7 mL</td>
<td>Shaking</td>
<td>87</td>
</tr>
<tr>
<td>Methanol</td>
<td>30 s</td>
<td>10 mg/2 mL</td>
<td>Vortexing</td>
<td>92</td>
</tr>
<tr>
<td>Methanol</td>
<td>30 min</td>
<td>—</td>
<td>Sonication</td>
<td>55</td>
</tr>
<tr>
<td>10 mM of HCl in methanol</td>
<td>60 min</td>
<td>100 mg/100 mL</td>
<td>Sonication</td>
<td>88</td>
</tr>
<tr>
<td>Methanol</td>
<td>15 min</td>
<td>100 mg/3 + 2 mL</td>
<td>Sonication</td>
<td>97</td>
</tr>
<tr>
<td>10% of acetic acid</td>
<td>8 h</td>
<td>0.05 g—0.5 g</td>
<td>Homogenization in a mortar</td>
<td>82</td>
</tr>
<tr>
<td>Methanol</td>
<td>12 and 24 h</td>
<td>25–100 mg/10 mL</td>
<td>Shaking</td>
<td>74</td>
</tr>
<tr>
<td>Methanol</td>
<td>3 × 24 h</td>
<td>1 g</td>
<td>Homogenization in a mortar.</td>
<td>69,101</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>3 × 30 min</td>
<td>10 g/3 × 100 mL</td>
<td>The suspension was left in</td>
<td>90</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1 h</td>
<td>20 mg/3 × 1 mL</td>
<td>methanol for 12 or 24 h</td>
<td>90</td>
</tr>
<tr>
<td>Water solution of acetic acid, pH 4, chloroform</td>
<td>1 h—maceration, 10 min</td>
<td>50 mg/1 mL</td>
<td>Sonication</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10–50 mg/10 mL</td>
<td>Maceration, extraction,</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>chloroform extraction</td>
<td></td>
</tr>
</tbody>
</table>
plates or silica gel plates.60,71,87,111–113 The methods of paper chromatography88 or bidirectional paper chromatography92,75 were also used for separation.

A method of psilocybin isolation using preparative thin-layer chromatography on silica gel plates followed by reextraction was developed.90

The method of isolation on cellulose sorbent was also elaborated followed by crystallization step.90

### Analytical Methods of Quantitative Determination

HPLC quantitative analysis of psychoactive substances originating from fungi of *Psilocybe* genus is usually performed by reversed-phase chromatography.84,87,92,98,101,102,104 Ion-pair chromatography applying reversed-phase column was also successfully used.87,98 As a method of determination, ion-exchange chromatography was also proposed using cation-exchange sorbent.85 Electrochemical detection is preferred due to low psilocin absorbance in UV light.84,114 Liquid chromatography (LC) combined with mass spectrometry (MS) is an effective method for detection of psilocybin and psilocin.92,102,104

Thermolabile compounds including psilocybin should not be analyzed directly by gas chromatography (GC) technique. The GC-MS analysis may be performed after derivatization with N-Methyl-N-(trimethylsilyl) trifluoroacetamide carried out to protect psilocin against thermal decomposition.93,103,105 Alternatively, electrospray ionization (ESI)–MS technique89,104,115,116 or chemical-type ionization117 may be used. Fluorescent psilocin derivative was detected by this ESI-MS technique.89 It is possible to detect psilocin O-glucuronide directly without earlier hydrolysis using LC-ESI-MS-MS technique.104

More sophisticated detection system based on chemiluminescence measurements was successfully used for psilocin determination.59,99

Ion mobility spectrometry may be an alternative method for rapid psilocin analysis.95

Capillary zone electrophoresis was also used for detection of psilocybin/psilocin and other indole compounds.55,97,118,119 A new method was developed for the screening of urine samples for the presence of 19 drugs of abuse including psilocin.106 The addition of β-cyclodextrin and organic solvents to the background electrolyte made possible selective separation of analytes.106

### Biologic Activity of Psilocybin/Psilocin in Mammalian Organisms

#### Psilocybin/Psilocin Pharmacokinetics

Psilocybin introduced to the digestive tract is very quickly hydrolyzed. Dephosphorylation under acidic environment of stomach or under activity of alkaline phosphatase in intestine leads to the appearance of active form of the drug, namely psilocin. Blockage of alkaline phosphatase by means of competitive substrates prevents the symptoms of intoxication.120 In opposition to psilocybin, psilocin is easily absorbed from the gastrointestinal tract to the circulating blood.121 Pharmacokinetic animal studies on 14C-labeled psilocybin analogue reveal that psilocybin is absorbed in 50% by the digestive tract after oral dose application.122,123 Similarly, HPLC studies revealed about 50% (52.7% ± 20%) bioavailability of psilocin after oral administration of psilocybin.114 Biodistribution of the drug after intravenous injection is similar for all organs including brain (animal study).122 After 8 hours, the tissue content of the drug is very low except for liver and adrenal tissue still expressing radioactivity after 48 hours.122

After oral application of psilocybin, plasma concentration of psilocin increases rapidly, reaches maximum after 50–100 minutes, and slowly decreases during the next 5–6 hours.54,114,124 Psilocybin injected intravenously rapidly undergoes dephosphorylation and disappears faster from circulatory system (when compared with oral administration).114 Pharmacokinetic parameters of psilocin after oral or intravenous administration of psilocybin determined in humans are shown in Table 2.

Animal studies reveal that psilocin is in 65% excreted with urine and in 15%–20% with bile and feces within 8 hours from administration.125 A small amount (about 10%–20%) remains in organism for a longer time (14C-labelled metabolites of psilocin have been identified in urine after 7 days from oral administration).122 About 25% of the whole dose was shown to be excreted unaltered in mice.122 Psilocin renal elimination profiles were studied also in humans.125 The psilocin excretion rate within 2–4 hours postdrug administration was equal to 55.5 ± 33.8 μg/h.125 Within 24 hours, only a small amount of the whole dose (mean 3.4%) was shown to be excreted as free psilocin.125 In the same study, it was demonstrated that after the treatment of urine samples with glucuronidase to measure the total psilocin concentration, the extent of psilocin excretion after 24 hours from administration increases only 2-fold (compared with free psilocin measurements). To explain this, it was proposed that hydroxyl groups of psilocin metabolites are also conjugated to O-glucuronide and in this form are excreted by kidneys.122 The glucuronidation is catalyzed by glucuronosyltransferases: microsomal enzymes originating from liver or intestinal tissues. After oral administration, psilocybin converted to psilocin is a substrate to UGT1A10 expressed by small intestine. After absorption within circulatory system the conversion of psilocin to glucuronide is mediated by UGT1A9.126 Other identified psilocin metabolites, namely 4-hydroxy-3-yl-acetic acid 4HIAA, 4-hydroxy-3-yl-acetaldehyde 4HA, and 4-hydroxytryptophol 4HT, are the products of its deamination and oxidation.114,122

<table>
<thead>
<tr>
<th>Psilocybin Dose</th>
<th>Way of Administration</th>
<th>$c_{max}$ (ng/mL)</th>
<th>$t_{max}$ (min)</th>
<th>$t_{1/2}$ (min)</th>
<th>$F_{abs}$ (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.224 mg/kg (10–20 mg)</td>
<td>Orally</td>
<td>8.2</td>
<td>105</td>
<td>163.3</td>
<td>52.7</td>
<td>114</td>
</tr>
<tr>
<td>1 mg</td>
<td>Intravenous</td>
<td>12.9</td>
<td>1.9</td>
<td>74.1</td>
<td>—</td>
<td>114</td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>Orally</td>
<td>6–21</td>
<td>70–90</td>
<td>135</td>
<td>—</td>
<td>84</td>
</tr>
</tbody>
</table>

© 2013 Lippincott Williams & Wilkins
Psilocin Pharmacodynamics

Psilocin as an alkaloid of the structure resembling serotonin molecular structure is able to bind and activate serotonin receptors. Chemical structures of psilocin, its precursor psilocybin, and serotonin are shown in Figure 1. Both physiologic and psychotic effects of psilocybin/psilocin intoxication result from its agonistic activity toward 5-hydroxytryptamine (5-HT) receptors located within or beyond the central nervous system.

Psilocybin should be considered as a prodrug. Product of its dephosphorylation, namely psilocin, as relatively lipophilic substance easily crosses blood–brain barrier.

Psilocybin/psilocin evoked effects are thought to be mediated mainly by activation of 5-HT2A receptor. In vivo studies in human subjects reveal that ketanserin, 5-HT2A receptor antagonist, prevents most of the psychotic symptoms usually developing after psilocybin/psilocin administration. However, some of the symptoms are not fully diminished and even intensify under combined psilocybin/ketanserin action (particularly cognitive deficits measured as difficulties in attention focusing and “reduction in vigilance”). The conclusion of the findings is that serotonergic system mediates psychotic effects, but they result from both stimulation of 5-HT2A receptors in cortical regions and activation of inhibitory 5-HT1A receptors located in dorsal raphe nucleus, leading to a decrease in tonic release of serotonin into prefrontal cortex.

From binding studies, it is known that psilocin as serotonin agonist binds with high affinity to 5-HT2A (Ki = 6 nM) and 5-HT2C (Ki = 10 nM) and with lower affinity to 5-HT1A receptor (Ki = 190 nM). Psilocybin and psilocin are known to inhibit some serotonin biologic activities dependent on 5-HT2C ligation, for example, serotonin-induced pruritus (after its topical administration). Similarly, N-methylated derivatives of psilocin and psilocybin (see Fig. 2), namely 1-methyl-psilocin and 1-methyl-psilocybin, exhibit comparable inhibitory action on serotonin activity. The 1-methylpsilocin selectively binds 2 isoforms of 5-HT2C receptor (Ki = 7.0 and 33 nM) and functionally activates the receptor (EC50 = 12 nM). The experimental data are in agreement with binding energies calculated from the data originating from computational prediction of the binding site structure in interaction with the studied derivatives. The N-methylated psilocin (Ki = 540 or >10,000 nM), similar to psilocin, inhibits serotonin-induced scratching. The 1-butyl psilocin (EC50 = 595 nM, Ki = 4.4 or 14 nM) was inactive in the behavioral test. The 4-fluoro-N,

N-dimethyltryptamine (Ki = 82 and 84 nM), despite relatively poor agonistic activity against 5-HT2C (EC50 = 90 nM), seemed to be able to inhibit serotonin-induced pruritus.

However, psilocin psychoactivity is mostly dependent on ligation of 5-HT2A receptors and inhibitory 5-HT1A receptors. Although 5-HT2A receptors are expressed by excitatory glutamatergic pyramidal neurons, the overall effect on neuronal activity is thought to be inhibitory due to the presence of 5-HT2A receptors also on GABAergic interneurons.

Triggered signaling pathways are different for various ligands of 5-HT2A receptor depending on ligand-induced conformational changes. Normally, serotonin stimulates phospholipase C releasing 2 important signaling molecules inositol 1,4,5-trisphosphate and diacylglycerol and leads to expression of transcription factor c-fos in a manner dependent on Gq/11 protein. Subsequent elevation of calcium ions leads to potassium channels closure and depolarization. Similarly, psilocin as serotonin agonist by binding to 5-HT2A receptor stimulates hydrolysis of phosphatidylinositol 4,5-bisphosphate. Stimulation of 5-HT2A receptor activates also phospholipase A2 and psilocin, but not its precursor psilocybin, and induces arachidonic acid release. More detailed investigations show that hallucinogenic serotonin agonists activate additional signaling pathways and induce transcription factors typically after activation of 5-HT1 receptor, although the receptor is not involved. It is also known that glutamate receptor designated mGluR2 forms functional complex with 5-HT2A receptor and modulates the serotonin receptor coupling to G protein, what implicates connection with psychosis induction and explains why ligation with hallucinogenic molecules results in the activation of specific signaling pathway. Activation of mGluR2 receptor breaks the signaling cascade and corrects symptoms of psychosis.

Ex vivo studies on rat brain showed that psilocin affects hippocampal pyramidal cells. Application of fungal extracts
containing psilocin and psilocybin decreases electrical activity of these neurons including activity stimulated by glutamic acid. 140

The activation of 5-HT1A receptor induces release of Ci00 protein subunits that activate potassium channel and inactivate calcium channel leading to hyperpolarization and inhibition of neuronal activity. Neurons expressing 5-HT1A receptors are highly responsive to psilocin action. Indeed, direct application of psilocin to neurons of dorsal raphe nucleus decreases frequency of neuronal firing, what suggests the involvement of 5-HT1A autoreceptor (its activation leads to serotonin decrease in synaptic cleft). 141

According to one of the existing hypotheses, the mechanism of waking dream is involved in visual hallucinations occurrence in course of psilocybin intoxication and in psychopathology of acute schizophrenic psychosis. 142 The state of waking dream is associated with low level of serotonin in brain. 143–145 During rapid eye movement phase, serotonergic neurons of dorsal raphe nucleus become inactive and their normal inhibitory activity on other brain areas transiently disappears. Similar phenomenon takes place under action of psilocin possibly due to stimulation of 5-HT1A autoreceptors present at higher density than other subtypes of 5-HT receptors on serotonergic neurons of raphe nuclei. 146,147

Psilocybin administration decreases binding of radio-labeled ligand of dopamine D2 receptors in striatum. The effect results from a rise of endogenic dopamine release and ligation. 148 It should be mentioned here that despite psilocin does not show any affinity to dopamine receptor of D2 type, modulating interactions between serotonergic and dopaminergic neuronal systems are known to exist.

Monoamine oxidase (MAO) inhibitors are taken by psilocin users to intensify its hallucinogenic effects. MAO is the enzyme catalyzing serotonin decomposition in synaptic cleft. Because psilocin may be MAO substrate, its presence causes inhibition of the enzyme. Brain serotonin is elevated and simultaneously 5-HIAA level decreases as a result of MAO blockage. 148

Early studies revealed that psilocybin elevates total level of ATP in central nervous system. 149 More detailed findings applying positron emission tomography determined elevated glucose metabolic rate under action of psilocybin. Increased metabolism of some cortical regions (particularly in anterior cingulate) and decreased metabolic activity in thalamus were observed after psilocybin administration in humans. 43,46

**Somatic and Psychotic Effects of Psilocybin/Psilocin Intoxication**

Dose dependent increases of blood pressure and heart rate were observed in course of psilocybin/psilocin intoxication. 13,15,150–152 The autonomic effects are maximal within 1–2 hours after drug administration. 150 Psilocybin elicits neuroendocrine effects similar to serotonin-induced changes in humans 150 (see Table 3).

Intensity of psilocybin intoxication depends on a dose of the drug. Psilocybin at a dose of 8–10 mg (0.1–0.2 mg/kg, orally) evokes only physiologic symptoms such as mydriasis, rarely hypotonic reaction associated with nausea and vomiting, slightly increased blood pressure and accelerated heart frequency, and paresthesias. 13,15,124,150–152,156 Pupillary dilation after administration of lower doses only increases sensitiveness to light, what should not be interpreted as psychotic perceptual changes. 156 However, subjects suffering from headaches are more sensitive to action of hallucinogens and undergo psychotic syndrome after intake of lower psilocybin doses. 157 Administration of higher doses (the amount of about 10–20 mg/70 kg, orally) leads to the elevation of plasma concentration of the drug up to the value of 5–12 ng/mL. 114 Psychotic syndrome starts to develop when psilocin plasma concentration reaches the value of about 4–6 ng/mL. 114 Psychotic syndrome is sometimes called schizophrenic phase. 146 The symptoms of the psychosis intensify during the next 30–60 minutes and start to decline after 2–3 hours. 13,50,114,150,151 Psilocybe semilanceata fruit bodies ingestion results in similar symptoms of intoxication including psychotic episode. 158 It is estimated that the consumption of hallucinogenic mushrooms in the amount of 2 g (about 10 fruit bodies of P. semilanceata) corresponding to 10–20 mg of psilocybin results in mild psychotic effect usually experienced as a pleasure (so-called “good trip”). The consumption of 12 g (about 60 specimens) corresponding to 60–120 mg of psilocybin is negatively experienced (as so-called “bad trip”). 159 The psychosis subsides after 5–6 hours from its appearance. The psychotic symptoms include disorders of visual perception such as illusions or hypnagogic experiences, alterations of time, color and motion perception (eg, kaleidoscopic vision), synesthesias, usually positively experienced sense of derealization and depersonalization, euphoria sometimes converting to drowsiness, anxiety, impaired mood, dysphoria, or emotional state described as extreme fear (mood swings). 15,46,48,128,151,153,160 Body image perception is affected—intoxicated subjects reported impression of swelling and numbness. 158 It seems that psilocybin impairs cognitive functions: decreases reaction time, impairs ability to focus and sustain attention mainly by producing strong unusual emotions and visions difficult to ignore, and thinking processes are disturbed by ego disorders and delusions. 41–50,128,129 Similar to schizophrenics subjects to whom psilocybin was administered have difficulties to use contextual information, what may be interpreted as cognitive deficit. 45 However, by lexical decision tasks, it is possible to show that psilocybin-induced psychosis, similar to schizophrenia-associated psychosis, predisposes to semantic network spreading. 50 Religious or sensual people may experience mystical feelings. 10–16 Personality characterized with specific curiosity (openness to cognitive or perceptual experimentation, also by means of psychoactive substances) and sensibility to a beauty of nature or works of art predisposes to pleasurable reactions (including those of mystical type). 163 At high doses, the schizophrenic phase is followed by the panic attack-like phase because pleasurable effects are replaced with dysphoria, paranoid thinking, and negatively experienced loss of ego control. 13,146,158 Low age and emotional excitability before the drug intake additionally predispose to such negative responses. 163 Accidental deaths resulted from jumping out of a window after the consumption of magic mushrooms were reported. 8,25 What suggests unbearable emotional state leading to suicidal attempt or a state of waking dream (flying is a common dream motif). The state of psychotic episode obtained after
### TABLE 3. Dose-Dependent Psychological and Somatic Effects of Psilocybin Treatment

| Psilocybin Dose Approximations of c_{max} Based on Data from114 | Plasma Concentration/c_{max} | Acute Psychological Effects (at the point of maximal drug effect, 50–150 min postdrug administration) | Subacute Psychological Effects | Long-Term Effects | Acute Somatic Effects, Measured Usually 1–2 h Postdrug Administration | References, Study Participants, Exclusion Criteria |
|---|---|---|---|---|---|---|---|
| 3–5 mg/70 kg (0.045–0.071 mg/kg) | Approximately 2 ng/mL | No increases in ASC scores | Headaches | No | No changes in cardiac electrophysiology, blood pressure, and heart rate tend to increase | Healthy, no personal or family history of psychiatric disorders, no history of drug abuse, usually had previous experiences with hallucinogens or cannabis |
| 8–10 mg/70 kg (0.115–0.143 mg/kg) | Approximately 5 ng/mL | OB, DED, VR, AA, RV, global ASC scores ↑, linearity between a dose and a mean score or number of subjects, desaturation, depersonalization phenomena | Working memory impairment | Fatigue headaches | Retrospective ratings: positive change to ASC—56%, in the relationship to the environment 38%, in aesthetic experiencing 37%, in the relationship to one’s body 30%, in awareness of personal problems 29%, in relationships to other 25%; concentration or memory problems were reported, complaints that required psychotherapy (rarely) | |
| 15 mg/70 kg (0.215 mg/kg) | Approximately 9 ng/mL | Drowsiness, increased sensitivity | | | Mood lability: harmony, happiness, grief, joy, anxiety, dysphoria, fear, paranoid thinking, subscale DED ↑ | |
| 20 mg/70 kg (0.315 mg/kg) | Approximately 14 ng/mL | Illusions, elementary and complex hallucinations, synesthesias, sensory alternations, alterations in perception of time and space, ASC VR ↑ | | | 90% of participants reported headaches; mean headache duration 16 h | |
| 30 mg/70 kg (0.429 mg/kg) | Approximately 19 ng/mL | Measurements done based on questionnaire considering: sense of unity, transcendence of time and space, sense of sacredness, sense of objective reality, positive mood, ineffability, paradoxicality, transiency; all ratings ↑ | | | Not measured | |
| Approximately 2 mg/kg, 90 mg/45–50 kg | Femoral venous blood 220 ng/mL (free psilocin), 4600 ng/mL (total psilocin); heart blood 60 ng/mL (free psilocin), 170 ng/mL (total psilocin); postmortem analysis | Possibly a state of waking dream (flying is a common dream motif), euphoria? | | | Death case | 55Accidental death case, death resulted from jumping out of a window after flying attempt |
TABLE 3. (Continued) Dose-Dependent Psychological and Somatic Effects of Psilocybin Treatment

<table>
<thead>
<tr>
<th>Psilocybin Dose</th>
<th>Subacute Psychological Effects</th>
<th>Long-Term Effects</th>
<th>Acute Somatic Effects, Measured Usually 1–2 h Postdrug Administration</th>
<th>References, Study Participants, Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg/70 kg (0.215 mg/kg)</td>
<td>Emotional excitation †, attention ability ↓</td>
<td>Working memory impairment</td>
<td>Fatigue, headaches</td>
<td>Arterial blood pressure †, heart rate †; prolactin ↑</td>
</tr>
<tr>
<td>20 mg/70 kg (0.315 mg/kg)</td>
<td>Emotional excitability †, anxiety, self-control, paranoid thinking, libido mood, primitive emotionality, emotional blunting</td>
<td>Impairment of thinking process, reaction time ↓, semantic priming promoted</td>
<td>AMRS scale “dreaminess” †, melancholia, efficiency-activation ↓, inactivation †, introversion †, extroversion ↓, fatigue, headaches</td>
<td>Arterial blood pressure †, heart rate †; prolactin ↑ and out of the normal physiologic range, thyroid-stimulating hormone ↑, adrenocorticotropic hormone ↑, cortisol ↑, γ-glutamyltransferase ↑, aspartate aminotransferase ↑</td>
</tr>
<tr>
<td>30 mg/70 kg (0.429 mg/kg)</td>
<td>Positive changes in mood, personal and, social behavior</td>
<td>Arterial blood pressure ↓, heart rate † (by about 10 b/min)</td>
<td>participant were male Protestant theology students, hallucinogen naïve, double-blind study (only in theory)</td>
<td>AMRS, Altman Mania Rating Scale.</td>
</tr>
</tbody>
</table>

Approximately 2 mg/kg, 90 mg/45–50 kg

ASC, the Altered States of Consciousness Rating Scale 5D consists of the following subscales: oceanic boundlessness (OB; measures positively experienced phenomena of derealization, depersonalization, and altered sense of time, elevated mood), dread of ego dissolution (DED; measures negatively sensed depersonalization and derealization, anxiety and cognitive deficits connected to ego disorders), visionary restructuring (VR; measures phenomena of elementary and visual (pseudo-)hallucinations, synesthesias, factors influencing imagination), auditory alterations (AA; measures disorders in auditory perception and hallucinations), reduction of vigilance (RV; measures impairment of alertness and associated disorders of cognitive functions).153

AMRS, Altman Mania Rating Scale.

even single psilocybin treatment may return particularly under action of other psychedelic substances such as alcohol or marihuana (so-called “flashback” effect).8,151,164

Psychological and dose-dependent somatic effects of psilocybin intoxication are shown in Table 3. Table 4 summarizes pharmacodynamic findings regarding psychotic properties of psilocybin.

Psilocybin/Psilocin Side Effects and Toxicity
The toxicity of psilocybin is low.165,166 For rodents, median lethal dose (LD50) equals to 285 mg/kg in mice and to 280 mg/kg in rats.165 Psilocybin administration results in transient elevation of blood concentration of some biochemical markers such as aspartate aminotransferase aspartate transaminase and γ-glutamyltransferase gamma-glutamyltransferase.150

In humans, even high doses able to induce psychosis (about 30 mg/70 kg, orally) only slightly and transiently affect heart rate and blood pressure without noticeable influence on heart function.13,15,150,152 Arterial pressure increase partly results from the mechanism controlling circulatory system involving central activation of 5-HT2 and 5-HT1A receptors. In addition, psilocin may express ligand activity toward 5-HT3A receptors placed on the surface of smooth muscle cells leading to vasoconstriction. Disturbances of blood clotting are possible.167 Rare cardiomyopathies have been reported: usually single cases of supraventricular tachycardia, myocardial infarction,56 and Takotsubo cardiomyopathy.168 Due to vasoconstrictor activity of serotonin agonists, the decreased blood flow through small arteries or blood vessels supplying nutrition to skin may lead to disturbances of circulation in lower and upper endings, formation of venous ulcers, and difficulties in wound healing. The risk of abortion resulted from vasoconstrictor activity of psilocin as serotonin agonist also exists. Postdrug administration transient headaches were also reported.151,154,158 Subchronic intoxication observed during 8 weeks of psilocin daily administration in rats leads to elevation of magnesium level in plasma, what likely composes protective mechanism against vasoconstrictor activity of psilocin.166

A destruction of blood–brain barrier under action of psilocin is possible.169 Similarly, serotonin released in high amounts from platelets may damage blood–brain barrier.170 The literature of the subject describes 1 case of 33-year-old patient who developed a monoparesis of the left leg and bilateral anopia 2 weeks after the consumption of magic mushrooms due to multifocal cerebral demyelination within the corpus callosum of the right cerebral hemisphere and both optic nerves.169

Potential Therapeutic Psilocybin/Psilocin Applications
Since the beginning of 1960s of XX century, the therapeutic potential of psilocybin has been a subject of scientific interest, particularly in psychiatry and psychotherapy. Harmfulness of the habit of psilocybin-containing mushrooms consumption, including all aspects of human health and risk for

© 2013 Lippincott Williams & Wilkins
### TABLE 4. Pharmacodynamic Findings Versus Dose-Dependent Psychological Effects of Psilocybin Treatment

<table>
<thead>
<tr>
<th>Psilocybin Dose Approximations of ( c_{\text{max}} ) Based on Data From114</th>
<th>( c_{\text{max}} )</th>
<th>Acute Psychological Effects (at the point of maximal drug effect, 50–150 min postdrug administration)</th>
<th>Pharmacodynamic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 mg/kg + placebo</td>
<td>Approximately 11 ng/mL</td>
<td>Increase in OB, VR and DED scores (about 45% vs placebo conditions)</td>
<td>Increase in reaction time (working memory deficits measured with visual manual delayed response task [DRT])</td>
</tr>
<tr>
<td>0.25 mg/kg + 20 mg ketanserin (5-HT2A antagonist)</td>
<td>Increases in OB, VR, and DED scores (about 20% vs placebo conditions)</td>
<td>Reduction of increase in reaction time</td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 20 mg ketanserin (5-HT2A antagonist)</td>
<td>No increase in OB, VR, and DED scores (vs placebo conditions)</td>
<td>No increase in reaction time</td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 0.021 mg haloperidol (D2 antagonist)</td>
<td>Increase in OB score (30%), increase in VR scores (40%); increase in DED score (65% vs placebo conditions)</td>
<td>Increase in reaction time</td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 0.5 mg risperidone (mixed 5-HT2 and D2 antagonist)</td>
<td>Increases in OB, VR, and DED scores (about 10%–20% vs placebo conditions)</td>
<td>Reduction of increase in reaction time</td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 1 mg risperidone (mixed 5-HT2 and D2 antagonist)</td>
<td>No increases in OB, VR, and DED scores (vs placebo conditions)</td>
<td>No increase in reaction time</td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td>Approximately 11 ng/mL</td>
<td>Derealization, loss of ego boundaries; OB ↑, VR ↑, DED ↑</td>
<td>Sensory perception alternations, illusions, complex scenery hallucinations, VR score ↑, RV ↑ (30%); attentional tracking reduced, spatial working memory not affected</td>
</tr>
<tr>
<td>0.215 mg/kg</td>
<td>Appr. 9 ng/mL</td>
<td>Increases in 5D-ASC scores: OB ↑ (40%); DED ↑ (20%); VR ↑ (50%); AA ↑ (15%); RV ↑ (30%)</td>
<td>RV ↑ (50%); attentional tracking reduced, spatial working memory not affected</td>
</tr>
<tr>
<td>0.215 mg/kg + 50 mg ketanserin (5-HT2A antagonist)</td>
<td>Increases in 5D-ASC scores: VR ↑ (15%); RV ↑ (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.115 mg/kg</td>
<td>Approximately 5 ng/mL</td>
<td>Increases in 5D-ASC scores: OB ↑ (10%); VR ↑ (15%)</td>
<td>Increase in VR score ↑ (15%). Binocular rivalry switch rate ↓, phase duration ↑ (from 2 s to about 2.7 s comparing to placebo conditions, measurement done 90 min after drug administration)</td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td>Approximately 11 ng/mL</td>
<td>Increases in 5D-ASC scores: OB ↑ (30%); DED ↑ (20%); VR ↑ (40%); AA ↑ (15%); RV ↑ (25%)</td>
<td>Increase in VR score ↑ (40%). Binocular rivalry switch rate ↓, phase duration ↑ (from 2 s to about 3 s comparing to placebo conditions, measurement done 90 min after drug administration)</td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td>Approximately 11 ng/mL</td>
<td>Increases in 5D-ASC scores: OB ↑ (40%); DED (20%) ↑; VR (50%) ↑; RV(30%) ↑</td>
<td>Binocular rivalry switch rate ↓, phase duration ↑</td>
</tr>
<tr>
<td>0.25 mg/kg + 50 mg ketanserin (5-HT2A antagonist)</td>
<td>Increase in 5D-ASC score RV ↑ (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.28 mg/kg</td>
<td>Approximately 12 ng/mL</td>
<td>Brief Psychiatric Rating Scale total score ↑: psychosis factor (conceptual disorganization, suspiciousness, hallucinatory behavior, unusual thought content) ↑; activation factor (tension, mannerism and posturing, excitement) ↑; anxiety/depression factor (somatic concern, anxiety, guilt feelings, depressive mood) ↑; anergia factor (emotional withdrawal, motor retardation, blunted affect) ↑; hostility factor (hostility, suspiciousness, uncooperativeness) ↑</td>
<td>Deficits in the AX-Continuous Performance Test (measure of visual context-dependent information processing), correct detection of AX sequences ↓, incorrect detection of BX sequences ↓</td>
</tr>
<tr>
<td>0.2 mg/kg + activation task (word association)</td>
<td>Approximately 9 ng/mL</td>
<td>Not measured</td>
<td>Difficulties in attention focusing, subjects found the activation task very difficult; number of associated and spoken words ↓</td>
</tr>
</tbody>
</table>

Stebelska Ther Drug Monit Volume 35, Number 4, August 2013
### TABLE 4. (Continued) Pharmacodynamic Findings Versus Dose-Dependent Psychological Effects of Psilocybin Treatment

<table>
<thead>
<tr>
<th>Psilocybin Dose</th>
<th>Approximations of (c_{\text{max}}) Based on Data From(^{114})</th>
<th>(c_{\text{max}})</th>
<th>Acute Psychological Effects (at the point of maximal drug effect, 50–150 min postdrug administration)</th>
<th>Pharmacodynamic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg for subjects &lt; 50 kg, 20 mg for subjects &gt; 50 kg + ketamine + (\beta)-amphetamine</td>
<td>Approximately 19 ng/mL</td>
<td>Derealization, depersonalization phenomena, loss of ego boundaries 3D-ASC scores ↑</td>
<td>Alternations in the perception of time and space, elementary and complex hallucinations, synesthesias</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Psilocybin Dose</th>
<th>Approximations of (c_{\text{max}}) Based on Data From(^{114})</th>
<th>References</th>
<th>Cognitive Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 mg/kg + placebo</td>
<td>Psilocybin-induced cognitive deficits and psychotic symptoms are dependent on activation 5-HT2A receptors</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 20 mg ketanserin (5-HT2A antagonist)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 40 mg ketanserin (5-HT2A antagonist)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 0.021 mg haloperidol (D2 antagonist)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 0.5 mg risperidone (mixed 5-HT2 and D2 antagonist)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 1 mg risperidone (mixed 5-HT2 and D2 antagonist)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td>Emotional disturbances, euphoria, anxiety, 4 subjects had high ratings in OB score, 3 subjects had high ratings in DED score</td>
<td>Difficulties in thinking, attention focusing</td>
<td></td>
</tr>
<tr>
<td>0.215 mg/kg</td>
<td>Psilocybin-induced activation of 5-HT2A receptors (leading to increased cortical stimulation) and activation of 5-HT1A receptors in raphe nucleus (leading to a decrease in tonic inhibitory serotonin release into prefrontal cortex), both effects impaired attention ability; activation of 5-HT1A receptors was responsible for the effect of reduced vigilance</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>0.215 mg/kg + 50 mg ketanserin (5-HT2A antagonist)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.115 mg/kg</td>
<td>Psilocybin-induced activation of 5-HT1A receptors affects brainstem oscillator functioning</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg + 50 mg ketanserin (5-HT2A antagonist)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(continued on next page)
TABLE 4. (Continued) Pharmacodynamic Findings Versus Dose-Dependent Psychological Effects of Psilocybin Treatment

<table>
<thead>
<tr>
<th>Psilocybin Dose Approximations of $c_{\text{max}}$ Based on Data From$^{144}$</th>
<th>Cognitive Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.28 mg/kg</td>
<td>Psilocybin psychotic action as 5-HT2A agonist is associated with context-dependent information processing, similar effect was observed in schizophrenics and in ketamine (NMDA antagonist) treated subjects, support for the hypothesis of glutamatergic neurotransmission disruption as a mechanism underlying psilocybin effects</td>
<td>45</td>
</tr>
<tr>
<td>0.2 mg/kg + activation task (word association)</td>
<td>Glucose metabolic rates: in right frontotemporal cortical regions $\uparrow$, particularly in the anterior cingulate $\uparrow$, in the thalamus $\downarrow$</td>
<td>45</td>
</tr>
<tr>
<td>15 mg for subjects $&lt;50$ kg, 20 mg for subjects $&gt;50$ kg + ketamine + d-amphetamine</td>
<td>Emotional lability, inability to control emotions, euphoria, dysphoria, paranoid thinking</td>
<td>Difficulties in attention focusing, thinking process disturbed with derealization and depersonalization phenomena</td>
</tr>
</tbody>
</table>

public health and safety, was recently estimated as very low.$^8$ However, anyone interested in the usage of hallucinogens for psychotherapy support should remember that seriously suffering patients would respond poorly to psilocybin and the drug could further negatively affect their mental health because it is able to produce schizophrenia mimicking psychosis.$^{41–50}$ Psilocybin at high doses ($20–30$ mg/$70$ kg) was shown to induce intense positive mystical-type experiences in healthy volunteers.$^{10–16}$ Participants of the mentioned studies on psilocybin-assisted psychotherapy self-reported improvements of quality of life after the drug session.$^{10–16}$ The results of the psychological experiments indicate positive influence of psilocybin-induced experiences on quality of social life including mood improvement and inclination toward more altruistic social behaviors. However, the acquired data mostly originating from questionnaire measurements (mystical scale questionnaire, eg) can be questioned taking into account the known, usually unpleasant physiologically and emotionally, symptoms of high-dose psilocybin intoxication. It is surprising that most of the participants confirmed mood improvement (regarded as an essential component of mystical experience) under high-dose psilocybin action. One explanation could be that they completed questionnaires 7 hours after drug ingestion (the whole session lasted 8 hours). After that time, all unpleasant effects of intoxication had already subsided. The study by Griffiths et al$^{13}$ was carefully designed to avoid expectancy effects as much as possible; however, the participants were recruited through announcements mentioning that the planned study aims to assess activity of “psychoactive substance used sacramentally in some cultures.” All study participants declared positive attitude toward religion and most of them were members of religious communities. Postdrug session mood improvements reported by participants of the experiment may be, at least partly, the effect of personal satisfaction resulted from strong engagement in absorbing scientific study confirming positive significance of religion and/or mystical experiences toward those they were well disposed.

Moreover, the study was designed in such a way that the participants were constantly monitored during drug session and also had an opportunity to talk with the observer. Therefore, when answering mystical scale questionnaire, they could be prompted by a suggestion, dishonest, or expressed metaphoric ideas (eg, having in memory and after the conceptual opposition death/resurrection). Communities conglomerated around religious cult are usually oppressive and their members are trained not only in conformity but also in cruelty by means of more or less sophisticated dangerous psychosocial techniques. Therefore, religiously inclined individuals, particularly if well educated, may mistake, intentionally or unknowingly, even basic emotions such as fear and pleasure. Moreover, religiously inclined people belonging to religious community are instructed to see any part of their emotional life as something positive and precious depending on intensity. It may lead to a kind rivalry, excessive emotional expression or exaltation, progressive incuriosity (emotional devaluation similar to drug addiction) and finally result in the discovery of being manipulated or forced for certain activities by cruel psychosocial or, like in this case, pharmacologic methods. Such a discovery is never satisfying (except for conformistic attitude) and may ruin system of values making impossible social adaptation, what takes place, for example, in course of schizophrenia. Indeed, about $40\%$ of participants of the mentioned study experienced fear or paranoid thinking during drug session.$^{13}$ Paranoid thinking included an impression of being manipulated or treated malevolently—some of the participants decided it is an occasion to inform about details on their private life.$^{13}$ Looking for intense, extraordinary emotions, preferably lacking
any connection with reality (escapism) is common to drug users and alcoholics. Their psychological profile is often characterized by emotional blunting—in both cases antisocial personality and aggression are more likely to develop in course of addiction than altruistic attitude. Similarly, as theatricalization of speech and behavior or myth-like confabulations (directly under action of alcohol or due to memory disorders in course of progressing the Korsakoff syndrome) in alcoholics, behavioral and mental changes developing under action of fungal hallucinogens may be directed against others, particularly if domination becomes the main purpose of the drug user. Religious context promotes reproduction of the behavioral pattern. Therefore, expecting positive influence of psilocybin treatment on quality of social life is simply ingenuousness. The authors of the mentioned studies remark that fear and paranoia developing under psilocybin action could be dangerous for intoxicated subjects. Moreover, they take into account possibility of long-term psychological effects of such experiences and try to measure them. Indeed, personality changes in adults after psilocybin single (!) treatment were demonstrated: particularly examined subjects became more open-minded. Such personality change should not be univocally interpreted as positive—it may reflect serious and dangerous ego disorders.

The therapeutic potential of psilocin and psilocybin was also tested in therapy of habitual criminals. One study (although critically reevaluated) demonstrated a short-term therapeutic effect observed as a decreased frequency of relapse into crime.171,172 There is also an increasing interest in therapy of anxiety neurosis, depression, and physical pain in patients with advanced-stage cancer by the use of psilocybin.36

In standard therapy, selective serotonin reuptake inhibitors are applied, but the results of such applications induce pruritus. In animal studies, the scratching of mice.171,172 efficacy of psilocin or to less extent psilocybin diminishes the itching effect in mice.171

Cluster headache, condition of unknown cause occurring periodically, is often resistant to therapy. Interestingly, psilocybin treatment results are promising. Examined patients treated with the drug-reported pain attack interruption and remission time extension.171

IBOTENIC ACID AND MUSCIMOL

Ibotenic Acid and Muscimol as Secondary Metabolites

Major active substances of Amanita muscaria and A. pantherina are ibotenic acid and muscimol—the products of the fungus secondary metabolism, thought to repel insects and slay their larvae. These 2 species are obligatory ectomycorrhizal and grow under deciduous and coniferous trees, especially beech, birch, and pine, in various types of woods. The mushrooms are cosmopolitan and occur wild in many continents (Europe, Africa, Asia, Australia, and America).62,178–180

Muscimol is a product of ibotenic acid decarboxylation. Both substances are polar and well soluble in water. Biochemical pathway of ibotenic acid and muscimol includes conversions of their precursor molecule β-hydroxyglutamic acid (ring closure and decarboxylation).180

According to some authors, the custom of the cuticle peeling off before mushrooms consumption aims to avoid the highest content of these psychoactive substances in the red cuticle and a yellowish flesh just under it.180 One of the pigments of muscaeaurin I (chemical structure shown in Fig. 3), being an aldimine derivative of ibotenic and betalamic acids.181,182 Apart from the poisonous properties, to both invertebrates and vertebrates, the pigment shows antioxidative potential and possibly protects against UV radiation. Quantitative analysis does not support the statement about higher content of ibotenic acid in the cuticle than in the flesh. Tsukijawa et al183 examined samples of A. muscaria and A. pantherina and found that isoxazole compounds tend to be more concentrated in the flesh than in the red cuticle. Ibotenic acid contents ranged from 10 to 2845 ppm in the cap of A. muscaria and from 188 to 269 ppm in the cap of A. pantherina.185 Muscimol was found in concentrations of 46–1052 ppm in the cap of A. muscaria and 1554–1880 ppm in the cap of A. pantherina.185 Another analysis of A. muscaria fruit bodies revealed similar ranges of concentration (for ibotenic acid, 182–1839 ppm in the cap and 627–1998 ppm in the stem and for muscimol, 46–1203 ppm in the cap and 82–292 ppm in the stem). Tsunoda et al185 found the ibotenic content ranging from 258 to 471 ppm and the muscimol content ranging from 18 to 27 ppm in A. muscaria samples. Stormer et al186 analyzed spores and fresh caps of fly agarics for the presence of ibotenic acid and they found the contents to be equal to 0.0054% and 0.0017%, respectively.186

Insecticidal Activity of Ibotenic Acid

The extract of A. muscaria fruit body is thought to be poisonous or overwhelming for flies. Glutamate and ibotenic acid are excitatory and inhibitory neurotransmitters in insects due to the exclusive presence of glutamate-gated chloride channels in invertebrates.187 Chloride channel activated by glutamate and ibotenic acid has been also identified in Drosophila melanogaster.188 By the measurement of hyperpolarizing response, the presence of an inhibitory glutamate receptor was demonstrated on the cell body membrane of a cockroach motor neuron.189 Inhibitory activity of ibotenic acid and other structurally similar compounds were shown on snail periodically oscillating neuron.190 Also insect muscle
fibers are responsible for ibotenic acid action. Ibotenic acid stimulates chloride channels distributed on the non-synaptic membrane of the coxal adductor muscle fibers but has no affinity for glutamate receptors located on the excitatory postsynaptic membrane. Inhibitory glutamate-gated chloride channels have been detected within plasma membranes of cockroach corpus allatum—densely innervated endocrine gland synthesizing juvenile hormone. Juvenile hormone controls processes of insect metamorphosis, vitellogenesis, and reproduction. Ibotenic acid was shown to be able to activate the channels what resulted in plasma membrane hyperpolarization. Stimulation of these inhibitory receptors is followed by reduction of juvenile hormone synthesis. Stimulation of excitatory glutamate receptors, which are also present on the surface of plasma membranes of corpus allatum, results in antagonistic effect on juvenile hormone production. Flies of Drosophila genus that normally feed on fly agaric fruit bodies seem to be resistant to fungal neurotoxins. Ibotenic acid and to lower extent muscimol are toxic to larvae of carpophageous species (D. melanogaster and D. immigrans).

Analytical Methods Used for Ibotenic Acid/Muscimol Detection and Isolation

Methods of Extraction

Muscimol is a product of ibotenic acid decarboxylation. Pure ibotenic acid is a crystal colorless substance (melting point of 150–152°C) readily soluble in water. Similarly, muscimol with the melting point of 175°C, although more hydrophobic, is well soluble in water. Curiously, pure ibotenic acid is extremely unstable in solution and rapidly decomposes to muscimol without involvement of enzymes. The addition of dimethyl sulfoxide to the water solution containing ibotenic acid reduces its stability. It was established that spontaneous decarboxylation of ibotenic acid is significantly accelerated in a mixture of dimethyl sulfoxide and D2O or D3O.

Ibotenic acid and muscimol were determined in fresh, frozen, or freeze-dried mushrooms. The following solvents are usually applied for extraction of ibotenic acid and muscimol: 75% methanol in water or 50%–70% ethanol in water. For briefly described methods of extraction used for ibotenic acid/muscimol analysis, see also Table 5.

Sometimes extraction to water acidified with formic acid is applied. It is thought that decarboxylation of ibotenic acid to muscimol takes place under these conditions. However, ibotenic acid was detected in water extracts containing formic acid. Methanol or ethanol extracts contain numerous lipid impurities that are possible to eliminate by several step extraction with nonpolar solvents.

Parapharmaceuticals, by producer declared to contain dried A. muscaria fruit bodies, were analyzed for the presence of ibotenic acid and muscimol applying modified the Stas-Otto extraction method commonly used for detection of psychoactive alkaloids. However, it seemed that the method was not sufficiently sensitive to detect A. muscaria isoxazoles in the examined material.

Isoxazole derivatives (carbamate in case of muscimol and carbamate and ethyl ester in case of ibotenic acid) were extracted to dichloromethane. Dansylated and ethyl ester derivative of ibotenic acid were separated from other components of the water–methanol extract by extraction of evaporated residue with ethyl acetate.

Methods of Qualitative Analysis and Isolation

Qualitative identification of ibotenic acid in water or water–ethanol extracts of pulverized A. muscaria fruit bodies is difficult due to the presence of interfering amino acid contaminations. Fluorescamine or more often ninhydrin were used for TLC detection. Detection is easier when bidirectional TLC is carried out in 2 different solvent systems. Preparative paper chromatography of extracts previously partly purified using ion-exchange chromatography was proven to be an effective method of ibotenic acid purification.

Amino acid contaminations may be eliminated by ion-exchange chromatography using cation- and anion-exchange sorbents. Crystalization step is necessary for satisfactory purification of ibotenic acid.

Analytical Methods of Quantitative Determination

Reversed-phase chromatography is the most often used HPLC technique to detect ibotenic acid and/or muscimol. Reversed-phase HPLC may be combined with ion-pair chromatography. Dansylated analytes were also separated using HPLC method on octadecyl column. Separation applying LiChrosor-NH2 and Nucleosil 5 CN columns was also effective for muscimol and ibotenic acid determination.

UV absorption–base detection system was used for dansylated derivatives quantification. For more detailed identification, eluent from diode detector was analyzed with mass spectrometer. GC–MS technique was used for detection of carbamate and ethyl ester derivatives of both isoxazoles in urine samples. Trimethylsilylation at 80°C for 30 minutes with N,O-Bis(trimethylsilyl)trifluoroacetamide.
results in conversion of isoxazoles to IBO-tri-TMS and MUS-di-TMS—both are possible to detect applying GC–MS. LC/MS/MS technique was used for detection of dansylated muscimol and dansylated ibotenic acid ethyl ester. LC/MS was also used for direct (without derivatization) detection of ibotenic acid in water extracts originating from fungal material and in urine samples.

**Biologic Activity of Ibotenic Acid and Muscimol in Mammalian Organisms**

**Ibotenic Acid/Muscimol Pharmacokinetics**

A minimal dose to obtain symptoms of central nervous system intoxication estimated for muscimol is 6 mg. In case of much less active but more dangerous ibotenic acid, the amount of 30–60 mg is sufficient for the psychodelic effect.

In contrast to γ-aminobutyric acid, muscimol is able to cross blood–brain barrier possibly by the mechanism of active transport or due to muscimol or its metabolite/derivative/adduct sufficiently high lipid membrane solubility. Early studies resulted in conflicting data regarding muscimol distribution within brain tissue and questioned the a priori accepted theory of gamma-aminobutyric acid (GABA) receptor population as the main binding site of muscimol. Muscimol was shown to be taken up by slices of cortex rat brain and the uptake was likely dependent on amino acid transport system localized in nerve terminals. The [3H]muscimol binding studies carried out using bovine brain GABA receptors reconstituted in lipid vesicles, it is known that muscimol binds to both high-affinity (Ka = 10 nM) and low-affinity (Kd = 0.27 μM) sites and functionally activates the receptor (EC50 = 0.2 μM). Wild-type rat GABA_A receptor subtype α1β2γ2 expressed in cell culture binds [3H]muscimol with Ka = 21–24 nM. Kinetic parameters of muscimol as GABA_A agonist were calculated also from functional assays (apparent affinity Kd = 10.9 μM, unbinding rate koff = 40/s; microscopic affinity constant Keq = 4.3 nM). Behavioral changes induced by muscimol in mice (sedation, ataxia) depend on its high-affinity binding in cerebral cortex and hippocampus to a distinct subtype of GABA_A receptors containing z6 subunit and lacking α1 subunit as shown in knockout study in mice.

The fast conversion of radiolabeled muscimol to other metabolites could be inhibited by pretreatment with an inhibitor of GABA-α-oxoglutarate transaminase. It has been also established with enzyme kinetics studies that muscimol is indeed an amine donor substrate to GABA transaminase. Another already identified metabolite of the fly agaric isoxazoles is tricholomic acid shown to be active in invertebrates as agent able to inhibit neurons. Tricholomic acid and other unknown ibotenic acid/muscimol metabolites may contribute to their activity.

Because in some cultures drinking urine originating from intoxicated subjects was practiced for ritual purposes, muscimol was often thought to be mostly unmetabolized. Indeed, both active compounds pass through organism quickly and partly unmetabolized. Ibotenic acid is in vivo metabolized to muscimol. The isoxazoles are excreted by kidneys. Both ibotenic acid and muscimol were detected in urine collected 3–8 hours after ingestion of poisonous mushrooms in humans (similar concentrations were determined despite different symptoms and stages of intoxication).

**Ibotenic Acid/Muscimol Pharmacodynamics**

Chemical structure of both isoxazoles closely resembles glutamic acid and a product of its enzymatic decarboxylation, namely γ-aminobutyric acid GABA (see Fig. 3). Structural similarity of ibotenic acid and muscimol to glutamic acid and γ-aminobutyric acid determines their ability to bind and activate receptors of the endogenous neurotransmitters.

Muscimol is an agonist of GABA_A and partial agonist of GABA_C receptors but evokes its activity mainly through GABA_A receptors ligation. From radioligand binding study using bovine brain GABA_A receptors reconstituted in lipid vesicles, it is known that muscimol binds to both high-affinity (Ka = 10 nM) and low-affinity (Kd = 0.27 μM) sites and functionally activates the receptor (EC50 = 0.2 μM). Wild-type rat GABA_A receptor subtype α1β2γ2 expressed in cell culture binds [3H]muscimol with Ka = 21–24 nM. Kinetic parameters of muscimol as GABA_A agonist were calculated also from functional assays (apparent affinity Kd = 10.9 μM, unbinding rate koff = 40/s; microscopic affinity constant Keq = 4.3 nM). Behavioral changes induced by muscimol in mice (sedation, ataxia) depend on its high-affinity binding in cerebral cortex and hippocampus to a distinct subtype of GABA_A receptors containing z6 subunit and lacking α1 subunit.

Additionally, muscimol inhibits GABA uptake by neurons and astrocytes and, as already mentioned, is a substrate to the GABA-metabolizing enzyme, namely GABA transaminase. Chemical structure of muscimol was used as a basis for the design of GABA uptake inhibitors and GABA agonists.

Ibotenic acid exhibits agonistic activity against N-Methyl-D-aspartic acid (NMDA) receptors and trans-1-Amino-1,3-dicarboxycyclopentane receptors (metabotropic quisqualate Qm receptors). However, neurotoxicity of ibotenic acid results only from activation of NMDA receptors. Activation of metabotropic Qm receptors followed by cellular signal cascade initiated by phosphoinositide hydrolysis is not involved in ibotenic acid–induced excitotoxicity. In vitro experiments revealed that ibotenic acid in contrast to kainic acid
acid does not suppress glutamate ligation and exhibits low affinity for sites binding radiolabeled kainic acid. Therefore, it is concluded that ibotenic acid is not a ligand of kainic receptors.

It is postulated that ibotenic acid and muscimol are the main compounds responsible for hallucinogenic properties of fly agaric and the mechanism of their toxicity results from ligation of glutamate and GABA receptors, respectively. High-dose muscimol treatment potentiates psychosis in schizophrenics. The subjects to whom muscimol was administered reported the experience of vivid dreams. However, the studies on the involvement of pedunculopontine tegmental nucleus in regulation of paradoxical sleep seem to negate hallucinogenic properties of GABA and glutamate agonists. Pedunculopontine tegmental nucleus is one of the brain areas involved in the process of paradoxical sleep appearance and various paradoxical sleep-associated phenomena. Injections of glutamic acid and muscimol into pedunculopontine tegmental nucleus induce suppression of one of the paradoxical sleep hallmarks, namely hippocampal theta rhythm in rats. However, it was also shown that muscimol locally administered to dorsal raphe nucleus increases REM sleep. The influence of both toxins on sleep architecture is likely the result of complex GABA and glutamate-dependent mechanisms modulating activity of cholinergic neurons within pedunculopontine tegmental nucleus and serotoninergic neurons of dorsal raphe nucleus.

Animal studies demonstrate that due to its inhibitory properties, muscimol reveals complex neuromodulatory activity. For example, muscimol (3 mg/kg, intraperitoneally) evokes serotonin rise and decreases catecholamine levels in brain. Muscimol applied locally was shown to decrease stratal GABA release in rats. The study by Naik et al revealed slight increase of the main catecholamine metabolite, homovanilic acid, in striatum after muscimol administration. Because muscimol alone decreases motor activity and muscimol coadministered with drugs such as cocaine prevents drug-induced motor hyperactivity dependent on dopamine neurotransmission, it is assumed that the isoxazole modulates dopamine release.

Muscimol, dose dependently, affects encephalogram in experimental animals distinctly from other typical hallucinogens such as lysergic acid diethylamide (LSD) and mescaeline. Particularly, electroencephalogram pattern elicited by muscimol consists of spikes, what characterizes substances of convulsant activity.

The intravenous administration of muscimol reduces glucose utilization throughout the central nervous system. However, the pattern of the effect was shown to be different from the known brain distribution of GABAergic neurons and GABA receptors. Another study revealed correlation between a decrease of cerebral glucose utilization and a broad fraction of muscimol (binding dependent on GABA<sub>A</sub> receptors). Decreased brain metabolism was associated with decreased cerebral blood flow. Muscimol lowers cyclic guanosine monophosphate level in cerebellum also in case of its elevation due to promoting convulsions activity of isoniazid.

Muscimol administered topically inhibits activity of spinal neurons in anaesthetized cats and the effect is diminished with bicuculline (potent GABA antagonist). In vitro experiments revealed that activation of GABA<sub>A</sub> receptors after ligation of muscimol induces presynaptic and postsynaptic suppression of synaptic transmission between muscle afferents and spinal cord motor neurons. Muscimol applied microiontophoretically decreased firing rate of cortical neurons.

Ibotenic acid is able to excite spinal interneurons and Renshaw cells as demonstrated in anaesthetized cats. The study reveled that excitatory activity of ibotenic acid is approximately 8 times higher than glutamate activity. However, after the recovery of excitation, the neurons became insensitive to the excitatory agents. The effect was abolished by bicuculline suggesting involvement of GABA<sub>A</sub> receptors. Therefore, it was concluded that ibotenic acid is in vivo decarboxylated to muscimol. Ibotenic acid (16 mg/kg, intraperitoneally) increases brain monoamines in rodents.

### Somatic and Psychotic Effects Resulted From Intoxication With *A. muscaria* and *A.a pantherina*

First symptoms of fly agaric intoxication apparent within 15–30 minutes after ingestion are signs of muscarinic poisoning such as nausea, vomiting, diarrhea, vasodilatation, sweating, and salivation. Then, after about 30 minutes from oral intake, atropine-like symptoms seem such as mydriasis, xerostomia, body temperature elevation, slightly increased blood pressure, drowsiness, amnesia, dizziness, hypersensitivity to light, euphoria, motor hyperactivity, hallucinations, and delirium. Mystical-type experiences were reported. The atropine-like symptoms intensify during the next 2–3 hours. Somnolence turns into deep sleep—the state is accompanied by skeletal muscles atony and tendon reflexes debility—the last symptoms may suggest stroke. Similar spectrum of intoxication (hallucinations, muscle twitching, delirium, sleep) has been observed in human volunteers after pure ibotenic acid and muscimol administration. Poisoning signs decline after about 8 hours. Somatic and psychotic effects observed in course of intoxication with poisonous mushrooms or pure muscimol and ibotenic acid are summarized in Table 6.

Within 30 minutes after intravenous injection of crude extract of *A. muscaria* changes in biochemical parameters have been observed in rats including decrease of acetylcholine esterase activity, decline of liver glycogen, decline of blood urea nitrogen, and increase of blood glucose. No changes of serum transaminase activity have been observed. After 6 hours, the parameters come back to the base line.

### Side Effects and Toxicity of Ibotenic Acid/Muscimol

Muscle twitching, spasms, cramps, and even seizures are frequent symptoms of fly agaric intoxication, particularly in children. Systemic or local administration of pure ibotenic acid results in convulsions as established also in animal studies.

Headaches and fatigue the next day after ingestion were reported. In case of ibotenic acid intoxication, headache may be prolonged up to a few weeks.

Lethal dose LD<sub>50</sub> of muscimol determined for rats is equal to 4.5 mg/kg after intravenous injection and 45 mg/kg after oral administration. The LD<sub>50</sub> established...
### TABLE 6. Somatic and Psychotic Symptoms of Intoxication With *Amanita muscaria* or *Amanita pantherina*—Mushrooms Containing Ibotenic Acid and Muscimol. Therapeutic and Side Effects of Muscimol

<table>
<thead>
<tr>
<th>Dose</th>
<th>Somatic Symptoms of Intoxication, Therapeutic, and Side Effects</th>
<th>Psychotic Symptoms of Intoxication</th>
<th>Long-Term Side Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal effective dose of muscimol: 6 mg; minimal effective dose of ibotenic acid: 30–60 mg; one fruit body of <em>A. muscaria</em> (50–70 g) may contain up to 70 mg of ibotenic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 fruit bodies of <em>A. muscaria</em> were eaten by 5 adults</td>
<td>Abdominal pain, nausea</td>
<td>Visual and auditory hallucinations; 18-year-old girl lost consciousness</td>
<td>Fatigue, stomach complaints the next day</td>
<td>58 Case report</td>
</tr>
<tr>
<td>5 caps of <em>A. pantherina</em> were eaten by 2 adults</td>
<td>2 h after ingestion: nausea, stomach-ache, diarrhea, vomiting; transiently increased blood pressure</td>
<td>2.5 h after ingestion: mania-like stimulation, agitation, mystical experiences, 4–5 h after ingestion the patient became more anxious, had difficulties to speak properly, reported vertigo, paresthesias of left arm</td>
<td>Disordered body perception, difficulties to speak properly</td>
<td>59 Case report</td>
</tr>
<tr>
<td>A 72-year-old patient ate a few fruit bodies of <em>A. pantherina</em></td>
<td>Diarrhea, vomiting</td>
<td>Neurologic disorders; hallucinations, coma, skeletal muscle flaccidity, hyporeflexia, facial asymmetry</td>
<td>Within 10 h after ingestion neurologic symptoms subsided</td>
<td>207 Case report</td>
</tr>
<tr>
<td><em>A. pantherina</em> poisoning case</td>
<td>Vomiting, urine analysis (4 h postinjection): ibotenic acid 47.4 µg/mL, muscimol 9.9 µg/mL</td>
<td>Hallucinations, the patient had difficulties in breathing</td>
<td>No long-term effects</td>
<td>202 Case report</td>
</tr>
<tr>
<td><em>A. pantherina</em> poisoning case</td>
<td>Normal blood pressure, regular heartbeat, urine analysis (8 h postinjection): ibotenic acid 32.2 µg/mL, muscimol 6.0 µg/mL</td>
<td>Dizziness, the patient was conscious</td>
<td>No side effects the next day</td>
<td></td>
</tr>
<tr>
<td><em>A. pantherina</em> poisoning case</td>
<td>Nausea, vomiting, normal blood pressure, regular heartbeat, urine analysis (3 h postinjection): ibotenic acid 37.3 µg/mL, muscimol 7.6 µg/mL</td>
<td>Hallucinations</td>
<td>No side effect the next day</td>
<td></td>
</tr>
<tr>
<td><em>A. pantherina</em> poisoning case</td>
<td>Slightly increased blood pressure, diarrhea, urine analysis (6 h postinjection): ibotenic acid 55.2 µg/mL, muscimol 7.4 µg/mL</td>
<td>Agitation, talkativeness, the patient described the psychotic effects as similar to alcoholic intoxication</td>
<td>No side effects the next day</td>
<td></td>
</tr>
<tr>
<td><em>A. pantherina</em> fruit bodies, portion consisting of about one half cup</td>
<td>No muscle spasms, no vomiting</td>
<td>90 min after ingestion, alternations of visual perception, wavy motions of static objects, altered perception of size and space, auditory hallucinations, impaired motor coordination, pleasurable emotionally altered state of consciousness lasting 7 h</td>
<td>No side effects</td>
<td>3 Self experimentation</td>
</tr>
<tr>
<td><em>A. pantherina</em> fruit bodies, portion consisting of about one half cup</td>
<td>Nausea</td>
<td>Drowsiness, sleep</td>
<td>No side effects</td>
<td>Case report</td>
</tr>
<tr>
<td><em>A. pantherina</em> fruit bodies, portion consisting of about a cup of fried mushrooms</td>
<td>—</td>
<td>Dissociative state (lasting 5 h), with no logical verbal contact with others, the subject experienced vivid waking dreams somehow related to the reality around him</td>
<td>No side effects</td>
<td>Case report</td>
</tr>
<tr>
<td><em>A. muscaria</em>, 30 g of dried caps</td>
<td>Muscarinic effects such as increased salivation and sweating, slight nausea</td>
<td>Symptoms developed 1 h after ingestion, pleasant sedation, alternations of visual perception, impaired motor coordination</td>
<td>No side effects</td>
<td>Self experimentation</td>
</tr>
</tbody>
</table>

(continued on next page)
in mice are 2.5 mg/kg (intraperitoneally), 3.8 mg/kg (subcutaneously), and 5.62 mg/kg (i.v.). The LD50 determined in rabbits is 10 mg/kg (orally). Lethal doses of ibotenic acid established in rats are 128 mg/kg (orally) and 42 mg/kg (intravenously). Ibotenic acid LD50 determined in mice equals to 15 mg/kg (intravenously) and 38 mg/kg (orally). To investigate an involvement of a particular brain area in the studied brain activity, ibotenic acid as a cytotoxic agent is directly injected into the brain tissue in vivo (animal experiments). Due to its cytotoxic properties, ibotenic acid is used to model the Alzheimer disease in rats. This compound injected into the entorhinal cortex causes neuronal losses via hippocampal perforant pathway resembling defects observed in the Alzheimer disease. Less toxic muscimol applied in similar experiments pharmacologically mimics cytotoxic agents by transient inactivation of neuronal activity. The mechanism of its action involves activation of GABA receptors followed by neuronal depolarization caused by chloride ions in flux. GABAA and GABAC receptors are GABA-gated ion channels specific for chloride ions. After ligation, they trigger inward chloride current, what inhibits neuronal activity. For example, it was established that microinjected muscimol inhibits the dorsomedial hypothalamus and the caudal region of the lateral/dorsolateral periaqueductal gray. The inactivation
was able to diminish autonomic responses (such as increases in heart rate and blood pressure, adrenocorticotropic hormone elevation) to stress conditions in rats.254

Potential Therapeutic Muscimol Applications

Due to its inhibitory activity, muscimol possesses analgesic properties. When injected intravenously, muscimol potentiates analgesic effect of pilocarpine in rats257 and morphine in rats and mice.255,256 All these effects are mediated by GABAergic system. Intrathecally injected muscimol, by GABA<sub>A</sub> agonistic activity, blocks nociception mediated by excitatory aminoacids.257 Muscimol was shown to potentiate anaesthetic activity of propofol, what allowed to establish GABAergic transmission involvement in the mechanism of anaesthesia induced by propofol.258

Intrathalamic microinjections of muscimol were able to reduce tremor in patients suffering from essential tremor, what would implicate therapeutic use of the isoxazole compound to treat the neurologic disorder.258

Muscimol applied systemically or topically to cortex was shown to block or delay drug-induced convulsions in animals.232,260 Pretreatment with muscimol (transmeningal administration) at 2.5 mM concentration prevents acetylcholine-induced seizures in rats and monkeys without serious side effects.261 Therefore, it could be potentially used for epilepsy treatment.

Moreover, despite psychoactive properties, muscimol applied in relatively low doses improves schizophrenia symptoms working as an anxiolytic likely by the mechanisms of neuronal activity suppression and/or paradoxical sleep suppression.221 In agreement with the finding of muscimol anxiolytic activity are results originating from later studies on the activation of GABA receptors localized in the lateral nucleus of the amygdala. Muscimol injections depending on concentration and the presence of selective antagonists affect fear learning and memory in rats. Particularly, activation of GABA<sub>A</sub> receptors by relatively low doses of muscimol in the presence of GABA<sub>C</sub> antagonist leads to fear learning blockade.262 Sedative effect of muscimol was also shown in mice.212,237

Muscimol was proven to improve tardive dyskinesia in schizophrenic patients.238,239

Muscimol is able to prevent cellular death in ischaemia–reperfusion conditions when released in high amounts glutamate exhibits cytotoxic activity. The preventive activity of muscimol was demonstrated in animal model of ischaemia–reperfusion.263,264 Muscimol stimulation of modulating hetero-receptor GABA<sub>A</sub> on glutamatergic neurons reduces activation of glutamate receptor NMDA, what protects pyramidal neurons against excessive excitability.265 The precise mechanisms of muscimol protective action is not known—it possibly involves inhibition of NMDA-dependent activation of neuronal nitric oxide synthase nNOS.266 In vitro studies revealed that GABA and muscimol inhibit NO synthesis in rat ileal nerve terminals through activation of GABA<sub>A</sub> and GABA<sub>C</sub> receptors.267 However, muscimol independently on its GABA<sub>A</sub> agonistic activity was also shown to stimulate NO release.268 Antiinflammatory activity of muscimol in endotoxemia was recently demonstrated in mice.268,269

CONCLUDING REMARKS

Psilocybin/psilocin referring scientific literature, including analytical chemistry studies, is quite rich. Therapeutic strategies based on psilocybin biologic activity are being developed. Agonists of serotonin receptors may find application in therapeutic areas such as psychiatry and neurology.4 The drugs of activity against serotonergic system such as ergotamine and sumatriptan are already used to treat migraine headaches.270,271 The results of search for potent and selective agonists of 5-HT<sub>2C</sub> receptors among psilocybin derivatives seem to be promising. On the other hand, psilocybin/psilocin–induced psychosis is commonly used to model schizophrenia, what allows for better understanding of its complex etiology and psychopathology.

Hallucinogens of fly agaric seem to be far less frequently a subject of chemical analysis and studies on the mechanisms of their biologic activity in humans. It is not surprising if one is aware of the use of A. muscaria isoxazole neurotoxins for a long time in neurobiology where ibotenic acid commonly serves as brain-lesioning agent. Less poisonous muscimol and its derivatives possess high therapeutic potential in neurology and psychiatry, although the potential is still insufficiently put to good account.

REFERENCES

17. Murrell T, Taylor W. The cutaneous reaction to nicotinic acid (niacin)-
18. Kobza Black A, Greaves MW, Hensby CN. The effect of systemic predi-
nisolone on arachidonic acid, and prostaglandin E2 and F2 alpha levels in
site of prostaglandin D2 release following oral administration of niacin
“flush” involves release of prostaglandins D2 from mast cells and sero-
tonin from platelets: evidence from human cells in vitro and on animal
sustained inflammation of human skin before and after aspirin. Clin Sci
significant differences in calcium-dependent phospholipase A2 activity
23. Messamore E. Relationship between the niacin skin flush response and
activity in schizophrenia with absent response to niacin.
25. Nagalski A, Bryka J. Natural history of jaundice: a clinical study in
North America. Arch Gen Psychiatry. 2003;60:1
27. Prochwicz K. Paradygmat poprzedzania semantycznego w badaniach
psychologicznych. Poznań: Wydawnictwo Uniwersytetu im. Adama
Mickiewicza; 2008. 311 p.
Nutrition and brain function. In: Siegel GJ,
29. Prochwicz K. Paradygmat poprzedzania semantycznego w badaniach
psychologicznych. Poznań: Wydawnictwo Uniwersytetu im. Adama
Mickiewicza; 2008. 311 p.
44. McMillan G, Blass JP. Nutrition and brain function. In: Siegel GJ,


169. Spengos K, Schwartz A, Hennerici M. Multifocal cerebral demyelin-


