Presence of Phenylethylamine in Hallucinogenic *Psilocybe* Mushroom: Possible Role in Adverse Reactions

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Abstract

The use of mushrooms containing the hallucinogenic substance psilocybin for intentional intoxication is relatively common. Occasionally, this results in adverse reactions with typical tachycardia that is not evidently caused by psilocybin. This study demonstrates the presence of phenylethylamine in the species *Psilocybe semilanceata* using gas chromatography–mass spectrometry and shows that the amount of this substance may vary much more than that of psilocybin. The highest amount of phenylethylamine (146 μg/g wet weight) was observed in mushrooms from a case of three young men hospitalized because of adverse reactions. Comparison of the symptoms observed in clinical cases of magic mushroom intoxication with those after intake of pure psilocybin or phenylethylamine suggests that phenylethylamine might have a role in the development of adverse reactions to *Psilocybe* mushroom intake.

Introduction

Magic mushrooms, which contain the hallucinogenic indole derivative psilocybin (Figure 1), are naturally occuring throughout the world. Abuse of hallucinogenic mushrooms is relatively common, and among young adults, it is one of the most frequently reported illicit drugs after cannabis (1–5). Apart from inducing drug-seeking behavior, such abuse entails a risk of adverse effects that require hospitalization. Pronounced adverse reactions after ingestion of psilocybin-containing mushrooms are reported to occur with a frequency of 13% (6). However, the exact mechanism of action and pharmacologic profile of pure drug compared with mushroom preparations remain to be fully elucidated (6,7).

Prison inmates discovered to be using magic mushrooms for intentional intoxication tested positive in the urine drug screening for the amphetamine class of compounds when a polyclonal antibody assay kit with a broad substrate reactivity was used. Because the assay does not detect psilocybin or its active metabolite, psilocin, the presence of an unknown compound structurally related to amphetamine was indicated. We therefore undertook this investigation of *Psilocybe semilanceata* (Liberty cap), which is the most common indigenous psilocybin-containing mushroom species.

Experimental

Chemicals

Phenylethylamine (PEA) hydrochloride was obtained from Sigma Chemical (St. Louis, MO) and psilocybin from Sandoz (Basel, Switzerland).
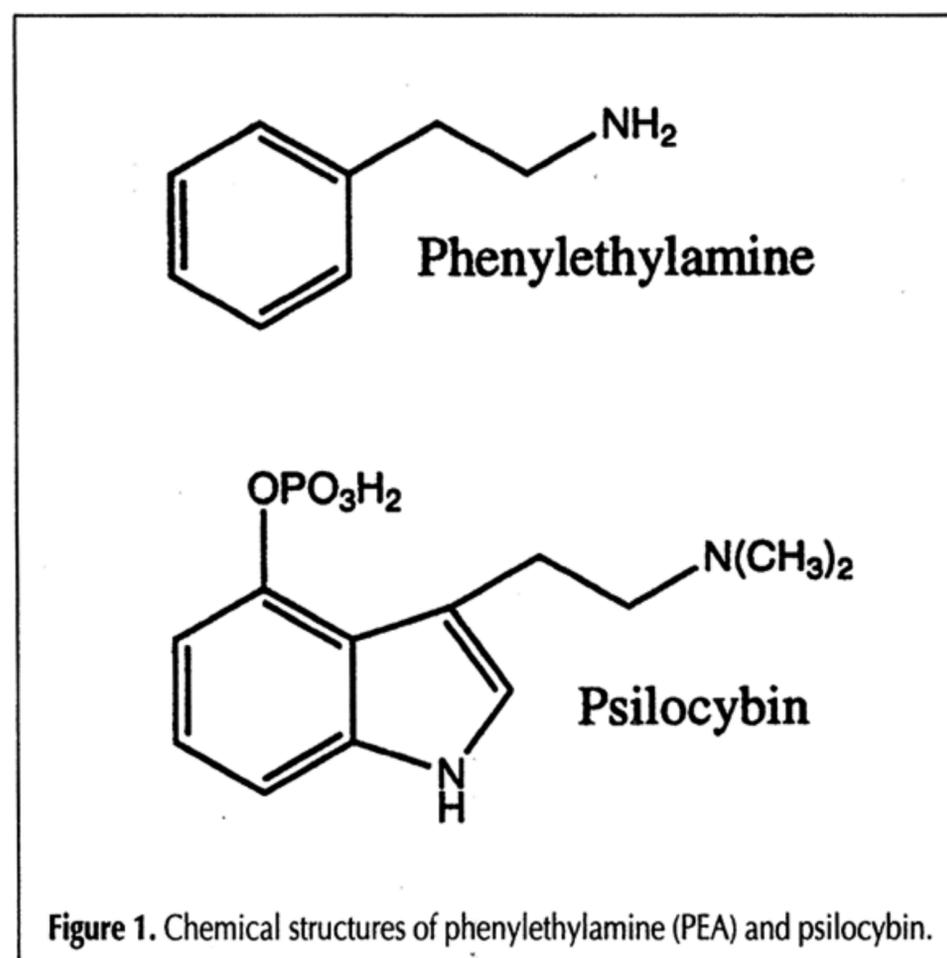

Figure 1. Chemical structures of phenylethylamine (PEA) and psilocybin.

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Specimens

Wild mushrooms were obtained fresh directly from different locations in Sweden and in connection with a case of three young men (ages 21–25 years) being hospitalized because of adverse reactions after eating magic mushrooms. The identity of the studied mushrooms as *Psilocybe semilanceata* was confirmed by macroscopic and microscopic inspection. Specimens were either stored refrigerated for up to one week and then transferred to plastic containers and stored in methanol at –20°C or air dried and stored in a closed container at ambient temperature. The specimens were prepared for analysis by disintegration of tissue in methanol (1–5 g mushroom/25 mL methanol) using an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany). Repeated freezing and thawing or sonication of tissue did not increase the recovery of substances (results not presented).

Chemical analysis

High-performance liquid chromatographic assay of psilocybin was performed on diluted (1000-fold with water) mushroom extracts using reversed-phase chromatography (150 × 4.0-mm Nucleosil C_{18}, 3 μm) with fluorescence detection (Shimadzu 551, Shimadzu Corp., Kyoto, Japan) and excitation and emission wavelengths at 270 and 330 nm, respectively. The mobile phase was composed of 10 mmol/L sodium phosphate, pH 3, with 6% (v/v) acetonitrile and 1% (v/v) methanol, and the flow rate was 1.0 mL/min. Aliquots of 20 μL were injected, and the detector response was linear in the concentration range used (0–1.4 mg/L). The intra-assay coefficient of variation (CV) was < 2% at a concentration of 0.24 mg/L.

Gas chromatographic (GC) analysis of PEA was performed using an HP 5890 GC (Hewlett-Packard, Palo Alto, CA) with a nitrogen-phosphorus detector (NPD) and a 25-m Chrompack WCOT CP-Sil 8CB fused-silica column (Chrompack Int. BV, Middelburg, The Netherlands) with a 0.25-mm internal diameter and 0.25-μm film thickness. Aliquots (10–500 μL) of the methanolic mushroom extracts were diluted with 3 mL phosphate buffer, and after addition of 0.6 mL 1 mol/L NaOH, they were extracted with 5 mL of 3% isopropanol in chloroform. The organic phase was extracted with 1 mL 0.1 mol/L H_2SO_4 and the aqueous phase transferred to new tubes. The extracts were alkalinized by addition of 0.2 mL 1.5 mol/L NaOH and extracted with 0.15 mL chloroform. Aliquots (1–2 μL) of the organic layer were injected into the GC system. The intra-assay CV was 5.0% at the level of 550 μg/L.

Gas chromatographic–mass spectrometric (GC–MS) analysis of PEA was performed using an ITS 40 WITNESS system (Finnigan MAT, San Jose, CA). Aliquots (10–100 μL) of the mushroom methanol extracts were diluted with 3 mL 0.1 mol/L sodium phosphate buffer, pH 6.0, and applied onto Certify Bond-Elut (Analytichem, Harbor City, CA) solid-phase extraction cartridges (prepared with methanol and 0.1 mol/L sodium phosphate buffer, pH 6.0). The columns were washed with 1 mL 1.0 mol/L acetic acid and 6 mL methanol and finally eluted with 2 mL ethyl acetate containing 2% ammonia. The
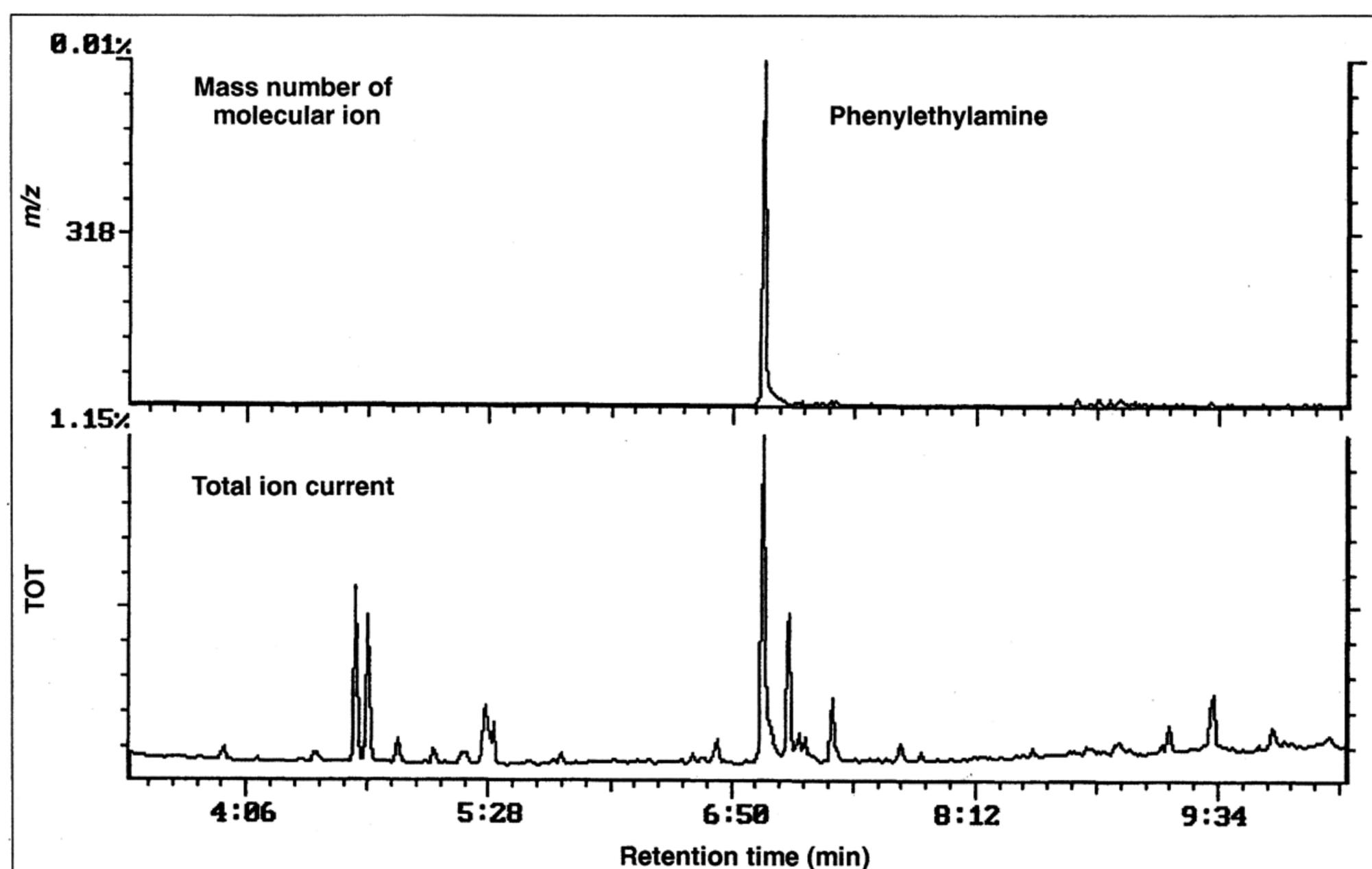

Figure 2. Total ion (lower trace) and selected ion (upper trace) chromatograms from the GC–MS analysis of a *Psilocybe* mushroom extract. The peak occurring at the retention time of phenylethylamine (PEA) is predominating. A peak at the same retention time is also seen in the trace for *m/z* 318 which corresponds to the molecular ion of PEA.

volume of the eluates were reduced to approximately 100 μL by evaporation, and the residue was treated with 70 μL heptafluorobutyric anhydride (Supelco, Bellefonte, PA) at 75°C for 60 min. After cooling, 0.5 mL ethyl acetate was added, and 1-μL aliquots were injected into the GC–MS system.

Clinical cases

Clinical data were collected from hospital case records sent to the Swedish Poisons Information Centre concerning *Psilocybe* mushroom poisoning during the period over 1980–1995. The total number of patients was 25, of which 21 were between 19 and 27 years of age. Five of the cases occurred in 1995.

Results

Chemical investigation

Psilocybin was detected in all mushrooms identified as *Psilocybe semilanceata*. The identification of PEA (Figure 1) in mushroom extracts was based on coelution with authentic compound in the GC system with components in underivatized form, and in the GC–MS system (Figure 2) with components in derivatized form. PEA was the major component seen in the chromatograms from both the GC and GC–MS analyses. In addition, mass spectral identification of PEA was performed after derivatization with heptafluorobutyric anhydride (Figure 3). The agreement of GC and GC–MS data with authentic compound confirmed the identity of PEA in the mushroom specimens.

PEA was readily detectable in all mushroom specimens. The amount of PEA was always less than that of psilocybin, but a much greater variability in PEA content was demonstrated (Figure 4). However, there was good agreement between samples collected in the same location and at the same time (see samples 2a–c in Figure 4). Sample 4 in Figure 4 contained the highest amount of PEA (146 μg/g wet weight) and came from a clinical case of hospitalization after the ingestion of magic mushrooms.

Clinical symptoms of intoxication

Recorded symptoms in hospitalized patients with adverse reactions to *Psilocybe* mushroom ingestion reported to the Swedish Poisons Information Centre are listed in Table I. The results are presented together with data compiled from the literature regarding clinical effects of mushroom ingestion as well as of pure psilocybin and PEA substances.

Discussion

The present study provides original evidence for the presence of the pharmacologically active substance PEA in *Psilocybe*
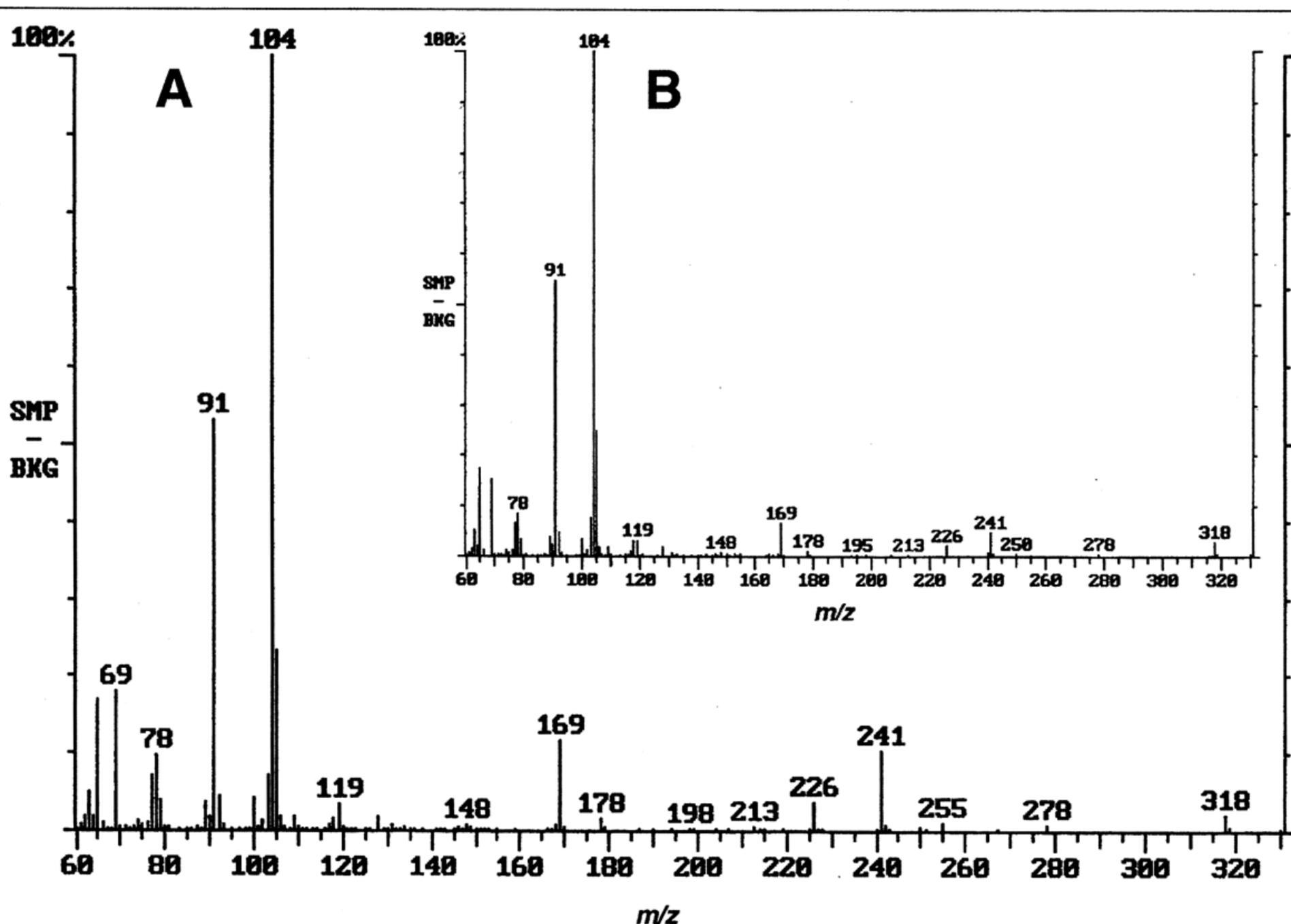

Figure 3. Electron impact mass spectra of unknown in mushroom extract (B) and reference phenylethylamine (A) as heptafluorobutyryl derivative.

semilanceata. PEA, the decarboxylated product of the ubiquitous amino acid phenylalanine, has received considerable interest within psychiatric research (8). Increased formation and excretion of PEA has been associated with psychosis in patients and implicated in the etiology of mental disease (9,10). Food is an exogenous source of PEA, which is present in chocolate, cheese, and wine, and has been implicated in the precipitation of dietary migraine (11). However, to our knowledge, PEA has not been previously identified in any species of mushroom.

The pharmacological mode of action of PEA is not fully elucidated, but it has been reported to exert amphetamine-like activity and to have peripheral sympathomimetic effects (6,7,12,13). The neurophysiological effects have been related to the enhancement of catecholaminergic activity (8). Systemic administration of PEA produces behavioral effects in rats and mice (14). The serotonergic system is thought to mediate the neurophysiological responses to hallucinogens (15,16). It is therefore interesting to note that serotonin receptor blockade can potentiate the behavioral effects of PEA (17). Although PEA resembles amphetamine structurally, it is seemingly not a substance with potential for abuse. However, despite substantial literature on the physiological functions of PEA, available data on its pharmacological effects in humans are more limited. In three reported cases in which PEA was unintentionally taken instead of amphetamine, the adverse reactions resulted in hospitalization (18). The symptoms observed in these specific cases are listed in Table I with our own and previously published clinical data from Psilocybe mushroom intoxications (19,20) and a controlled experiment using pure psilocybin substance (21). There are appreciable differences between the symptoms observed after psilocybin ingestion and mushroom intoxication. Visual hallucinations and euphoria occur less frequently after mushroom ingestion, which might be explained by a bias in selection of only those patients admitted to the hospital. However, tachycardia was observed in only 1 of 14 volunteer subjects given pure psilocybin, and this small influence on the heart rate has been observed by others (22,23). In contrast, tachycardia is a common finding in patients intoxicated by *Psilocybe* mushrooms, an observation that also points toward a role for PEA in the adverse effects of such mushroom ingestion.

Within the human body, PEA is formed at a high rate, but it is also rapidly inactivated through metabolic breakdown by monoamine oxidase (MAO), which leads to the formation of phenylacetic acid (8). PEA is metabolized mainly by the MAO-B isozyme, which is found in serotonergic regions of the human brain (24). Ingested psilocybin readily becomes dephosphorylated to psilocin, which is further inactivated by MAO, leading to formation of 4-hydroxyindoleacetic acid (25). However, this has been shown in the rat to be a minor metabolic pathway, which indicates that psilocin is a poor substrate for MAO. Whether psilocin is a MAO-A or B substrate has not been clarified, but the effect of its structural analogue *N,N*-dimethyltryptamine (DMT) is

Table I. Principal Clinical Features Following Ingestion of *Psilocybe semilanceata* Authentic Psilocybin, and Phenylethylamine (PEA)

Signs and symptoms	*Psilocybe* mushroom		Psilocybin	PEA
	25 patients*	71 patients[†]	14 volunteer subjects[‡]	3 patients[§]
	n (%)	*n* (%)	*n* (%)	*n* (%)
Visual hallucinations	13 (52)	53 (75)	12 (86)	–
Euphoria	6 (24)	9 (13)	10 (72)	–
Anxiety	10 (40)	41 (58)	9 (64)	2 (67)
Agitation	4 (16)	14 (20)	–	–
Mydriasis	17 (68)	60 (85)	14 (100)	–
Tachycardia	11 (44)	20 (28)	1 (7)	3 (100)
Hypertension	4 (16)	18 (25)	2 (14)	–
Hyperreflexia	3 (12)	28 (39)	–	–
Flushing	3 (12)	11 (15)	4 (29)	–
Nausea, vomiting	3 (12)	21 (37)	6 (43)	3 (100)
Flashbacks	2 (8)	2 (3)	–	–

*Summary of hospital case records at the Swedish Poisons Information Centre in 1980–1995.
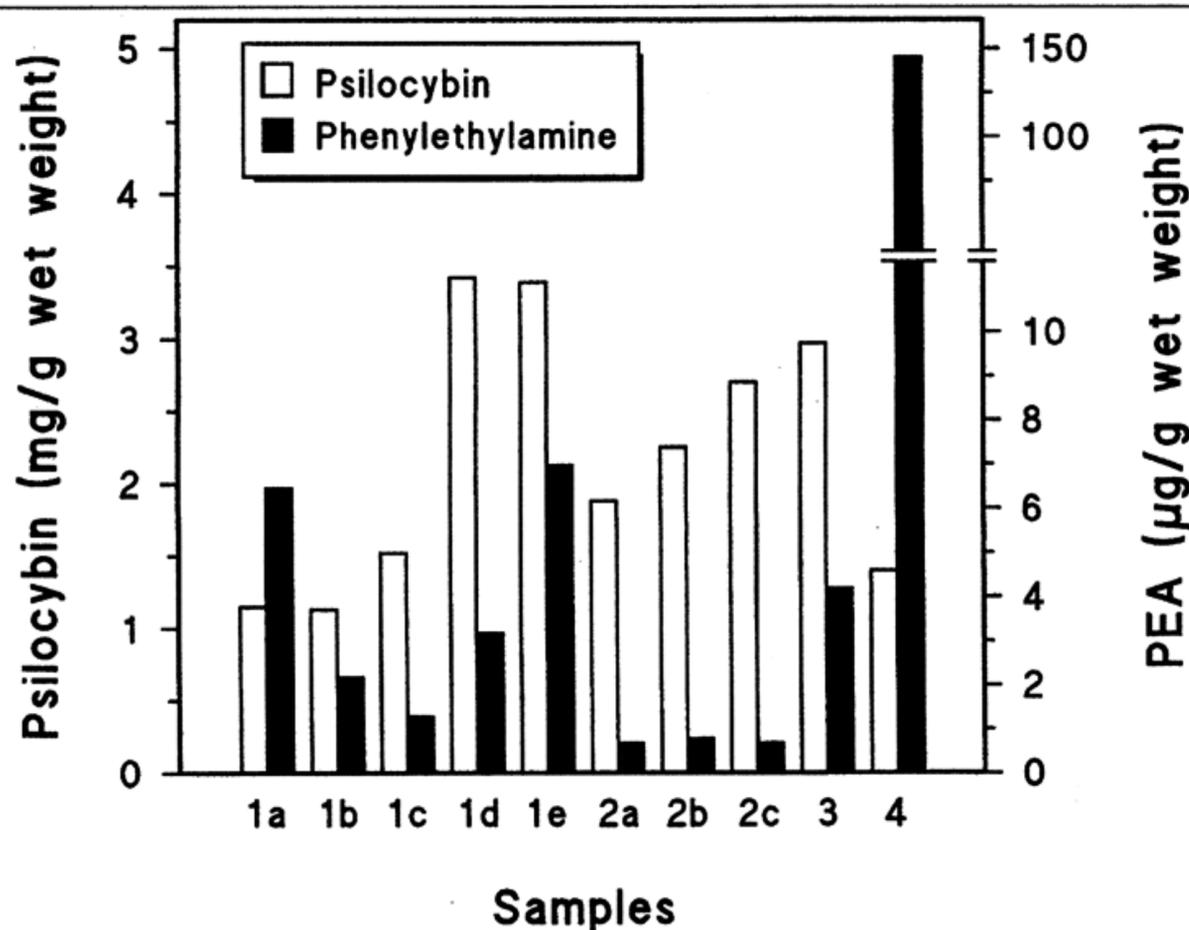[†] References 19 and 20.
[‡] Reference 21.
[§] Reference 18.

Figure 4. Psilocybin and phenylethylamine (PEA) levels in *Psilocybe semilanceata* mushrooms collected at four different locations (1–4) in Sweden. Multiple collections from a single location are indicated by alphabetic notation (a–e). Sample 4 was obtained from a case of mushroom intoxication that led to adverse reactions and hospitalization.

greatly enhanced by the coingestion of MAO inhibiting substances (26). Thus, although speculative, a metabolic interaction between PEA and psilocin through competitive inhibition of MAO is possible.

The presence of PEA in a common species of hallucinogenic mushrooms may have implications for drugs-of-abuse testing because we have occasionally detected PEA in urines testing positive for the amphetamine class of compounds by immunoassay technique (unpublished observation). As a consequence, ingestion of magic mushrooms should be considered in such cases. Today, *Psilocybe* mushroom abuse is generally not screened for in drugs-of-abuse testing despite the reports of common usage (1–5). Using reagent kits sensitive to PEA and including psilocin in the verification assay might offer a means of detecting this kind of drug abuse.

In conclusion, we have shown that PEA is readily present in the hallucinogenic mushroom species *Psilocybe semilanceata*. The high amount of PEA observed in mushrooms obtained from one case of intoxication suggests that PEA may contribute to the adverse reactions. The much higher variability in PEA content as compared with psilocybin is intriguing because it could explain why adverse reactions occur only in certain cases. Future studies will need to determine the factors influencing PEA formation in *Psilocybe semilanceata* and determine if PEA is present in other common hallucinogenic mushroom species being used for intoxication.

Acknowledgments

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