Effect of Psilocin on Extracellular Dopamine and Serotonin Levels in the Mesoaccumbens and Mesocortical Pathway in Awake Rats

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Psilocin (3-[2-(dimethylamino)ethyl]-1H-indol-4-ol) is a hallucinogenic component of the Mexican mushroom Psilocybe mexicana and a skeletal serotonin (5-HT) analogue. Psilocin is the active metabolite of psilocybin (3-[2-(dimethylamino)ethyl]-1H-indol-4-yl dihydrogen phosphate). In the present study, we examined the effects of systemically administered psilocin on extracellular dopamine and 5-HT concentrations in the ventral tegmental area (VTA), nucleus accumbens, and medial prefrontal cortex of the dopaminergic pathway in awake rats using in vivo microdialysis. Intraperitoneal administration of psilocin (5, 10 mg/kg) significantly increased extracellular dopamine levels in the nucleus accumbens. Psilocin did not affect the extracellular 5-HT level in the nucleus accumbens. Conversely, systemic administration of psilocin (10 mg/kg) significantly increased extracellular 5-HT levels in the medial prefrontal cortex of rats, but dopamine was decreased in this region. However, neither extracellular dopamine nor 5-HT levels in the VTA were altered by administration of psilocin. Behaviorally, psilocin significantly increased the number of head twitches. Thus, psilocin affects the dopaminergic system in the nucleus accumbens. In the serotonergic system, psilocin contribute to a crucial effect in the medial prefrontal cortex. The present data suggest that psilocin increased both the extracellular dopamine and 5-HT concentrations in the mesoaccumbens and/or mesocortical pathway.

Key words psilocin; dopamine; serotonin; nucleus accumbens; ventral tegmental area; medial prefrontal cortex

Native peoples in Mexico have used Psilocybe mushrooms throughout history in religious ceremonies and for healing. Naturally occurring hallucinogenic substances such as psilocybin and mescaline have been recognized for their ability to alter perception, cognition, and emotion.1,2 Both psilocin and psilocybin are hallucinogenic components of the Mexican mushroom Psilocybe mexicana and are serotonin (5-HT) analogues. Psilocin is the active metabolite of psilocybin and is considered a pharmacologically active species.3,4 A number of previous studies have examined the hallucinogenic effects of psilocybin in human volunteers.5–9 Psilocybin produces changes in mood, disturbances in thinking, illusions, and complex visual hallucinations in healthy human volunteers.9 Moreover, preliminary clinical trials have determined that psilocybin is effective at reducing the symptoms of obsessive-compulsive disorder10 and is an effective anxiolytic agent in terminal cancer patients with anxiety.11

Recent pharmacological studies suggest that the hallucinogenic effects of psilocin and psilocybin are produced mainly because these compounds act as 5-HT receptor agonists that bind 5-HT 1A, 5-HT 2A, 5-HT 2B, and 5-HT 2C receptors with moderate to high affinity.12,13 The effects of psilocybin are blocked by pretreatment with the 5-HT 2A receptor antagonist ketanserin, indicating that the hallucinogenic effects of psilocybin are mediated primarily by 5-HT 2A stimulation.14–16 Thus, 5-HT 2A receptor activation is necessary for the indoleamine hallucinogenic effects of psilocybin. On the other hand, a human imaging study using radiolabeled raclopride, a dopamine (D 2) receptor antagonist, showed reduced binding in the caudate and putamen after psilocybin administration, suggesting that psilocybin indirectly increases dopamine levels in the brain, probably through 5-HT 2A receptor activation.17

Functional interactions between central dopamine and 5-HT systems have been well documented. Electrophysiological, biochemical, and behavioral evidence suggest that the ascending serotonergic pathways from the medial and dorsal raphe modulate or control the function of the mesoaccumbens and mesocortical dopaminergic system.18,19 Here we studied the effects of psilocin on dopamine and 5-HT transmission in the ventral tegmental area (VTA), nucleus accumbens, and medial prefrontal cortex, which are structures responsible for perception, cognition, and mood. The present study using in vivo microdialysis that was designed to evaluate extracellular dopamine and 5-HT levels in these regions.

MATERIALS AND METHODS

Surgical Procedures Male Wistar rats weighing 250 to 300 g were used. Experiments were carried out in accordance with the guidelines for animal care of Showa Pharmaceutical University (No. P-2014-2), as well as the guidelines for animal use published by the National Institutes of Health.

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Rats were anesthetized with pentobarbital-Na (50 mg/kg, intraperitoneally (i.p.)) and positioned in a stereotaxic apparatus. Guide cannulae in only one brain area per rat were implanted into the upper part of the VTA (anterior–posterior: −5.0 mm; lateral: 0.5–0.6 mm; depth: −6.5 to −7.5 mm), nucleus accumbens (anterior–posterior: 1.6 mm; lateral: 1.6–1.8 mm; depth: −5.2 mm), or medial prefrontal cortex (anterior–posterior: 3.0 mm; lateral: 0.6 mm; depth: −3.0 mm) according to the atlas of Paxinos and Watson. All experiments were performed 7 d after surgery.

**Microdialysis Technique** Microdialysis probes (dialysis membrane: length, 2.0 mm; diameter, 0.3 mm; MW cut-off, 2000 Daltons; AF-01, Eicom Co., Kyoto, Japan) were inserted into the nucleus accumbens, VTA, or medial prefrontal cortex through the implanted guide cannulae. The animals were placed in a Plexiglas cage (30 cm×30 cm×38 cm), and the cannulae were connected by polyethylene inflow and outflow tubes to a syringe pump and collection vials. Perfusion solution (125 mM NaCl, 3 mM KCl, 1.3 mM CaCl₂, 1 mM MgCl₂, and 23 mM NaHCO₃) in aqueous potassium phosphate buffer was perfused into each dialysis probe at a rate of 2 µL/min. Perfusion samples were collected at 20-min intervals. After three baseline samples were collected, physiological saline or psilocin was intraperitoneally injected, and sampling continued to the end of the experiments. We also simultaneously observed psilocin-induced behavior. Dopamine and 5-HT were quantitatively measured in each perfusate sample using high-performance liquid chromatography (HPLC) with electrochemical detection. The HPLC system (HPLC-ECD, HTEC-500 system, Eicom Co.) and conditions were as follows: Eicompak SC-5ODS column (3.0 mm i.d.×150 mm) with a pre-column; mobile phase, 85% 0.1 M citric acid/0.1 M sodium acetate buffer, 15% methanol, 0.023% sodium 1-octanesulfonate, containing 5 mg/L disodium—ethylenediaminetetraacetic acid (EDTA); flow rate, 500 µL/min; electrode, Eicom WE-3G graphite electrode; reference electrode, Eicom RE-100 Ag/AgCl; applied voltage, 650 mV vs. Ag/AgCl. The column temperature was maintained at 25°C.

**Head-Twitch Test** The head-twitch test was performed as described by Rojas-Corrales et al., with minor modifications. Rats were transferred to the testing room 30 min before testing. Immediately after psilocin administration, the rats were placed into an observation cage (30 cm×25 cm×18 cm), and the cumulative number of head twitches (rapid paroxysmal movements of the head with little or no involvement of the trunk) was counted for 60 min by an observer blinded to the study protocol. The test cage was thoroughly cleaned between animals.

**Data Evaluation** The effects of psilocin on extracellular dopamine and 5-HT concentrations are presented as the mean percentage±S.E.M. of the baseline value, which was calculated from three consecutive samples taken immediately prior to drug administration. The significance between the groups was analyzed with repeated measurement two-way ANOVA, followed by Dunnett’s multiple comparison test. For analysis of two groups, the significance of the difference was analyzed using the F-test, followed by the Student’s or Aspin–Welch’s t-test. A value of p<0.05 was considered significant.

**Materials** Psilocin as an oxalic acid salt was synthesized in the Department of Medicinal Chemistry of Showa Pharmaceutical University. Psilocin was prepared for intraperitoneal administration of Psilocin (10 mg/kg, n=5) significantly increased the number of head twitches during a 60-min observation period. Data are expressed as the means±S.E.M. (bars) and as the number of head twitches during a 20-min period. Asterisks indicate statistical significance (**p<0.01) vs. the saline-treated group with Dunnett’s multiple comparison test.

**Nucleus Accumbens**

**A: Dopamine**

- Control
- Psilocin 1 mg/kg
- Psilocin 5 mg/kg
- Psilocin 10 mg/kg

**B: 5-HT**

- Control
- Psilocin 1 mg/kg
- Psilocin 5 mg/kg
- Psilocin 10 mg/kg

Psilocin (5, 10 mg/kg, n=5–6) significantly increased extracellular dopamine levels in a dose- and time-dependent manner in the nucleus accumbens. Asterisks indicate statistical significance (*p<0.05, **p<0.01) vs. the saline-treated group with Dunnett’s multiple comparison test.

Psilocin (1, 5, 10 mg/kg, n=5–6) did not affect extracellular 5-HT levels in the nucleus accumbens. Data are expressed as means±S.E.M. (bars) and as percentages of baseline values. Baseline levels of dialysate dopamine and 5-HT were 2.20±0.4 and 0.48±0.1 nM, respectively.
administration by dissolving the hydrochloride salt in saline.

RESULTS

**Effect of Intraperitoneal Administration of Psilocin on Behavioral Responses** Systemic administration of a low dose of psilocin (1 mg/kg, i.p., n=4) did not induce changes in behavior. Higher doses of psilocin (5 and 10 mg/kg, i.p., n=5–6) reduced locomotor activity, investigatory behavior, and sniffing after injection. However, 10 mg/kg psilocin induced a significant increase in the number of head twitches during a 60-min period (Fig. 1).

**Effect of Intraperitoneal Administration of Psilocin on Extracellular Dopamine and 5-HT Concentrations in the Nucleus Accumbens** Intraperitoneal administration of psilocin caused a dose-dependent increase in dialysate dopamine concentrations in the nucleus accumbens. Psilocin at doses of 5 and 10 mg/kg but not 1 mg/kg caused a significant increase in extracellular dopamine levels relative to those of the saline treatment group. Maximal increases of 118.5±9.2% (p<0.05) at 20 min after administration with 5 mg/kg and 139.1±12.7% (p<0.01) at 40 min after administration with 10 mg/kg were observed (Fig. 2A). Extracellular dopamine levels returned to baseline levels within 140 min after systemic psilocin (10 mg/kg) administration. In contrast, administration of saline or psilocin (1, 5, 10 mg/kg) did not significantly affect extracellular 5-HT levels (Fig. 2B).

**Effect of Intraperitoneal Administration of Psilocin on Extracellular Dopamine and 5-HT Concentrations in the VTA** We examined the effect of psilocin on extracellular dopamine and 5-HT concentrations in the VTA. As shown in Fig. 3, intraperitoneal administration of psilocin (10 mg/kg) did not significantly increase dopamine levels in the VTA during the 180 min following administration (Fig. 3A). Similarly, psilocin did not increase the 5-HT level in the VTA (Fig. 3B).

**Effect of Intraperitoneal Administration of Psilocin on Extracellular Dopamine Concentrations in the Medial Prefrontal Cortex** Intraperitoneal administration of psilocin (10 mg/kg) decreased the dopamine levels during the 60–80 min following administration (p<0.05, Fig. 4A). In contrast, administration of psilocin increased the 5-HT concentrations in the dialysate in the medial prefrontal cortex (p<0.05, Fig. 4B).

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**Fig. 3.** A) Effect of Intraperitoneal Administration of Psilocin on Extracellular Dopamine Levels in the VTA
Psilocin (10 mg/kg, n=5) did not affect extracellular dopamine levels in the VTA. B) Effect of Intraperitoneal Administration of Psilocin on Extracellular 5-HT Levels in the VTA
Psilocin (10 mg/kg, n=5) did not significantly affect extracellular 5-HT levels in the VTA. Data are expressed as the means±S.E.M. (bars) and as percentages of baseline values. Baseline levels of dialysate dopamine and 5-HT were 0.25±0.1 and 0.33±0.2 nM, respectively.

**Fig. 4.** A) Effect of Intraperitoneal Administration of Psilocin on Extracellular Dopamine Levels in the Medial Prefrontal Cortex
Psilocin (10 mg/kg, n=5) induced a small but significant decrease in extracellular dopamine levels 60–80 min after administration in the medial prefrontal cortex. Asterisks indicate statistical significance (*p<0.05, **p<0.01) vs. the saline-treated group with Dunnett’s multiple comparison test. B) Effect of Intraperitoneal Administration of Psilocin on Extracellular 5-HT Levels in the Medial Prefrontal Cortex
Psilocin (10 mg/kg, n=5) significantly increased extracellular 5-HT levels in the medial prefrontal cortex. Data are expressed as the means±S.E.M. (bars) and as percentages of baseline values. Baseline levels of dialysate dopamine and 5-HT were 0.20±0.2 and 0.31±0.1 nM, respectively.
Fig. 4B). Psilocin at 10mg/kg induced maximal increases in 5-HT of 151.9±6.9% (p<0.01) 20–40 min after drug administration. 5-HT levels in the dialysate returned to baseline within 80 min after systemic psilocin administration (Fig. 4B).

DISCUSSION

Psilocin is a potent hallucinogen, producing profound psychological and perceptual alterations in humans at relatively low doses (50–100µg/kg). With oral administration of psilocin, the half-life ranges between 2 and 3 h, and peak levels of psilocin appear between 80–105 min. A recent mouse study showed that systemic administration of psilocin (0.3–4.8mg/kg) reduces locomotor activity, rearing, and investigatory behavior, and increases the head twitch response. Consistent with this previous study, we observed that psilocin (5, 10mg/kg) administration produced a decrease in locomotor activity and rearing and an increase in the head twitch response.

The major finding of the present study is that intraperitoneal administration of psilocin (5, 10mg/kg), the hallucinogenic component of magic mushrooms, significantly increased extracellular concentrations of dopamine but not 5-HT in the nucleus accumbens. On the other hand, systemic administration of 10mg/kg psilocin did not increase extracellular dopamine, but did increase 5-HT levels in the medial prefrontal cortex of awake rats. Regarding dopamine release, some studies using microdialysis have shown that local administration of 5-HT or 5-HT agonists facilitates extracellular dopamine release in the nucleus accumbens. Moreover, 5-HT1A agonists may facilitate dopamine release in the nucleus accumbens, whereas 5-HT2A antagonists have been reported to inhibit dopamine release in the nucleus accumbens. Likewise, psilocybin decreases [3H]raclopride binding in the caudate nucleus and putamen in humans, suggesting that psilocybin indirectly increases the striatal dopamine concentration. These data are consistent with the findings in our study. Psilocin may increase dopamine levels in the nucleus accumbens through concomitant stimulation of both 5-HT1A and 5-HT2A receptors.

The present results show that psilocin did not increase extracellular dopamine, but did increase 5-HT levels in the medial prefrontal cortex. Several studies indicate that psilocin acts as an agonist at 5-HT1A, 5-HT2A, and 5-HT2C receptors. Furthermore, 5-HT2A/C receptors are expressed in the rodent prefrontal cortex. The hallucinogenic 5-HT2A/C agonist 4-iodo-2,5-dimethoxymethamphetamine indirectly increases extracellular 5-HT release in the rat medial prefrontal cortex by stimulating glutamate release. Most of the subjective effects of psilocybin are blocked by pretreatment with the 5-HT2A/C antagonist ketanserin, indicating that the hallucinogenic effects of psilocybin are mediated primarily by actions at the 5-HT2A receptor. We hypothesize that psilocin increases extracellular 5-HT levels through 5-HT2A receptor activation in the mesocortical pathway.

Interestingly, we found that psilocin was associated with a modest decrease in the level of dopamine in the medial prefrontal cortex 60–80 min after administration. 5-HT1A and 5-HT2A receptor agonists facilitate the release of dopamine in the brain, whereas 5-HT2C receptor agonists inhibit dopamine release. Systemic administration of 5-HT2C receptor agonists causes a decrease in dopamine release in the prefrontal cortex, whereas antagonists increase dopamine release in the prefrontal cortex. Regulation of mesocortical dopamine by cortical 5-HT2C receptors may involve a polysynaptic neuronal circuit, and activation of 5-HT2C receptors may regulate mesocortical dopamine levels. We speculate that psilocin may decrease dopamine release in the prefrontal cortex through activation of 5-HT2C receptors but not 5-HT1A or 5-HT2A receptors.

In conclusion, our data suggest that the important effect of psilocin is to increase the level of dopamine and 5-HT in the mesoaccumbens and/or mesocortical pathway.

Conflict of Interest The authors declare no conflict of interest.

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