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Mushrooms from genus Clitocybe and Tricholoma as potential new sources of CNS-active agents

Grzyby z rodzaju *Clitocybe* i *Tricholoma* jako potencjalne nowe źródła czynników aktywnych wobec CUN

INTRODUCTION

Disorders, illness and disease of central nervous system (CNS) are among the most common ailments affecting mankind. It has been estimated, for some populations in the world, that above 30% of people suffer from disorders of the brain once in a lifetime at least, and above 10% of others suffer a mental disorders at least monthly. The use of natural products from various sources, including fungi, as psychotropic agents to alleviate CNS disorders, or to create altered mental states (also in therapeutic purposes), probably is as old as the dawn of mankind, when the first trial-and-error experiments by shamans and witch-doctors took place. Some other experiments led to the discovery of effects of certain plant and fungal extracts on the mind. Although there is extensive literature on the use of fungi and fungal extracts as hallucinogenic and stimulating agents, the search for natural products for therapeutic psychotropic purposes is much less well-developed than, for example, the search for antitumor or antiatherosclerotic agents [1, 7]. From some sources from Italy, where mushroom clouded agarics *Clitocybe nebularis* called "nebbiolini" is consumed in big quantities, we had information, that it is also called "madness mushroom", because it causes nausea while cooking of big quantities of product for processing (oral, ethnic information). Similar information was obtained from ethnic sources about other mushrooms from genus *Clitocybe* and *Tricholoma* in some regions of Poland. Our article is an analysis of new species of mushrooms as potential new sources of CNS-active substances.

MATERIAL AND METHODS

Mushroom materials and extractions. Fruit bodies of the mushrooms from genus *Clitocybe* and *Tricholoma* were collected in forests near Lublin. Extraction procedures with ethanol,

methanol, cold water and hot water, and TLC analysis of active substances were performed according Gross [2] and Stijve et al. [5]. Psilocybin and psilocin derivatives for standards were obtained from Sigma-Aldrich, USA.

Analysis of CNS – stimulating activities. Analyses of 5HT receptors agonism and antagonism potency, of obtained extracts, were performed according Tadipatri et al. [6] with modifications by Zjawiony and Lewellyn (personal communication). Control levels of serotonin receptors were standardized according National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP).

RESULTS AND DISCUSSION

During various type of extractions of the mushrooms from genus *Clitocybe* and *Tricholoma*, we isolated substances with R_f close in TLC to some psilocybin derivatives (Table 1), like aeruginascin or baeocystin [3, 5]. It indicates that the mushrooms from genus *Clitocybe* and *Tricholoma* can be treated as new "magic mushrooms" with CNS-stimulating activities. It means that they contain substances useful as psychotropic agents to alleviate CNS disorders, or to create altered mental states (in therapeutic purposes). We found very interesting stimulation (agonists) or inhibition (antagonists) of 5HT5A and 5HT2C receptors, and NET transporters, by extraction of these mushrooms (Table 2). For 5HT5A receptors, there are completely new natural substances. Only synthetic ones have been known until now [1]. It means that they are very promising fungi for potential therapeutic, psychotropic purposes, maybe sometimes atypical [1, 4, 7]. Experiments on purification and more detailed identification of bioactive substances from these mushrooms are in progress.

Table 1. R_f of main spots obtained in TLC analysis, of extracts of the fungi from genus *Clitocybe* and *Tricholoma*, in butanol-acetic acid-water system (1) and propanol-ammonia system (2). Extraction procedures was performed according to Gross [2], and TLC analysis was performed according to Stijve et al. [5]

Mushroom	TLC system	Methanol Extracts	Ethanol Extracts	Cold water Extracts	Hot water Extracts
Clitocybe nebularis	1	0.42	0.42	0.31	0.31
	2	0.25	0.30	0.17	0.18
Clitocybe irina	1	0.44	0.44	0.21	0.22
	2	0.26	0.29	0.18	0.19
Clitocybe odora	1	0.44	0.43	0.22	0.22
	2	0.26	0.27	0.17	0.19
Tricholoma sulphureum	1	0.40	0.41	-	-
	2	0.26	0.24	-	-
Tricholoma saponaceum	1	0.43	0.44	0.22	0.23
	2	0.27	0.27	0.15	0.16
Tricholoma pardinum	1	0.41	0.41	-	-
	2	0.28	0.29	-	-

Table 2. Agonist (+) and antagonist (-) potency of extracts of the fungi from genus *Clitocybe* and *Tricholoma*, against 5HT2C and 5HT5A receptors and NET transporters. The activity was measured, as described in Materials and Methods. Controls (100%) of serotonin receptors were standardized according NIMH PDSP

Mushroom	Receptor	Methanol	Ethanol	Cold water	Hot water
		Extracts			
		(% of inhibition [-] or stimulation [+] of appropriate receptor)			
Clitocybe nebularis	5HT5A	+6.0	-3.7	+50.7	+56.0
	5HT2C	+6.0	-5.9	+44.6	-5.0
	NET	-	+8.4	+7.7	+58.1
Clitocybe irina	5HT5A	-	-	+30.0	+22.7
	5HT2C	-	-	+10.0	+19.5
	NET	-	-	-	-
Clitocybe odora	5HT5A	+4.4	-4.3	+22.2	+9.7
	5HT2C	+9.0	-6.8	-	-
	NET	-	-	-	-
Tricholoma sulphureum	5HT5A	+8.0	-4.1	-	-
	5HT2C	+7.0	-6.3	-	-
	NET	-	-	-	-
Tricholoma saponaceum	5HT5A	-	-	+5.5	+3.8
	5HT2C	-	-	+11.0	+1.6
	NET	-	-	+5.0	+33.9
Tricholoma pardinum	5HT5A	-	-	+4.7	+2.1
	5HT2C	-	-	+1.0	+5.9
	NET	-	-	-	-

CONCLUSIONS

In this study we described extraction of new bioactive substances with CNS-stimulating activities, from the fungi from genus *Clitocybe* and *Tricholoma*. We found they are either agonists or antagonists of 5HT5A and 5HT2C receptors, and NET transporters. We found that the extracts contain completely new natural organics active against 5HT5A receptors.

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SUMMARY

Novel CNS-stimulating activities were extracted from the fruit bodies of mushrooms from genus *Clitocybe* and *Tricholoma*. Fractions active against 5HT5A and 5HT2C receptors, and NET transporters were extracted with methanol, ethanol, cold water and hot water. Their 5HT receptors agonism and antagonism potency were characterized against appropriate receptor proteins. It indicates that the fungi from genus *Clitocybe* and *Tricholoma* can be treated as new "magic mushrooms" with CNS-stimulating activities. And these organics are very promising bioactive substances. It means that these mushrooms are a very promising source for potential therapeutic and psychotropic purposes, also.

Key words: CNS-stimulating mushrooms, 5HT receptors agonist, 5HT receptor antagonist, *Clitocybe* sp., *Tricholoma* sp.

STRESZCZENIE

Z owocników kilku grzybów z rodzajów *Clitocybe* i *Tricholoma* wyekstrahowano nowe aktywności stymulujące CUN. Frakcje aktywne względem receptorów 5HT5A i 5HT2C oraz transporterów NET wyekstrahowano przy użyciu etanolu, metanolu, zimnej i gorącej wody. Ich potencję jako agonistów i antagonistów receptorów 5HT scharakteryzowano używając odpowiednich białek receptorowych. Otrzymane wyniki sugerują, że izolowane substancje z owocników kilku grzybów z rodzajów *Clitocybe* i *Tricholoma* pozwalają je traktować jako nowe „grzyby magiczne" stymulujące CUN. Te substancje organiczne są bardzo obiecującymi związkami bioaktywnymi, a zawierające je grzyby z rodzajów *Clitocybe* i *Tricholoma* także posiadają potencjalne zastosowania terapeutyczne i psychotropowe.

Słowa kluczowe: agonista receptora 5HT, antagonista receptora 5HT, *Clitocybe sp.*, *Tricholoma sp*.