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Studies on Psilocybin and related compounds

I. Communication

Structure/activity relationship of oxyindole-derivatives with regard to their effect on the knee jerk of spinal cats

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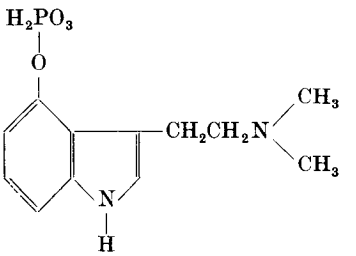
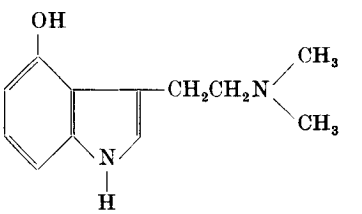
(2 Figures)

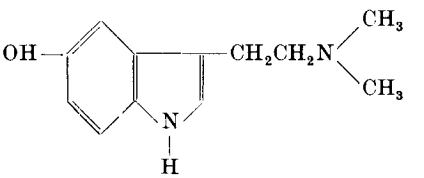
The active ingredients of several hallucinogenic mexican mushrooms, psilocybin and psilocin have been identified as tryptamine derivatives, psilocin being an isomer of bufotenin [1].



Psilocybin



Psilocin



Bufotenin

The outstanding chemical feature of these new indoles is the 4-position of the hydroxygroup, which previously was not known among the many natural indole-derivatives. In the course of our pharmacological investigations of psilocybin and psilocin [2] we have found that both compounds—i.e. independently whether the hydroxygroup of the indole ring is conjugated with phosphoric acid or not—can sharply be distinguished from the 5-oxy-

indole-derivatives serotonin and bufotenin by the different effect on spinal reflexes [3]. There are undoubtedly other criteria, which also allow to differentiate the Psilocybe-agents from serotonin and bufotenin as for example the lack of psilocybin to stimulate in the anesthetized cat intrathoracic receptors leading to a reflex fall in blood pressure. Also the socalled tryptamine receptors of some smooth muscle organs react very different to psilocybin and serotonin:

Whereas any serotonin-like action of psilocybin, for example on the isolated rat uterus, is still absent, the effect of subsequently applied serotonin is blocked. Psilocybin is therefore rather to be classified among serotonin antagonists than among serotonin-like substances.

With regard to the pronounced psychotropic activity of psilocybin in man, pharmacological tests in vivo are preferable, especially if in addition at least some functions of the central nervous system are involved in the test procedure. Special attention was therefore paid to the phenomenon that psilocybin causes, like LSD, an increased excitability of spinal reflexes. This action, as observed in animals, has also been verified in human tests, both for LSD and psilocybin [4, 5, 6].

The testing of the influence of psilocybin and serotonin on the knee jerk of spinal cats proved to be a highly sensitive as well as a good discriminating method. Both compounds show an effect already in the dose range of 5 to 10 μg/kg i.v. with psilocybin enhancing, but serotonin blocking the reflex. A comparative study was therefore carried out with some 30–40 tryptamine compounds. Most of them were synthetized only recently by *Troxler, Seemann* and *Hofmann* [7] as derivatives of psilocybin and psilocin. Our study aims to establish some correlation between chemical structure on one side and type and intensity of action of the respective substances on the other side.

Method

Cats of both sexes, weighing between 2 and 3 kg were used. Under ether anesthesia the spinal cord was transected between the first and second vertebra and the brain destroyed. The knee jerk was elicited by blows of an automatically driven hammer, delivering a constant mechanical stimulus every two seconds. The reflex extension of the hind limb was recorded with a semi-isometric lever on a smoked drum. Carotid blood pressure was continuously measured on a mercury manometer. Serotonin was injected in form of the creatininsulfate, whereas the other substances were used as free bases. All injections were made intravenously (injection time 20 seconds) through an indwelling canula in one of the jugular veins using the following sequence of doses: 5, 10, 20, 50, 100, 200 μg/kg. The single reaction obtained was not evaluated quantitatively according to the intensity and/or duration of the effect. Instead we determined for each substance the minimal i.v. dose, which produced a distinct and clearly visible stimulation or inhibition on the continuously recorded reflex activity as exemplified by fig. 1 and 2. For testing each substance up to 8 animals were used. Only in cases where even with 200 μg/kg no alteration at all was observed, the number was limited to 2 animals.

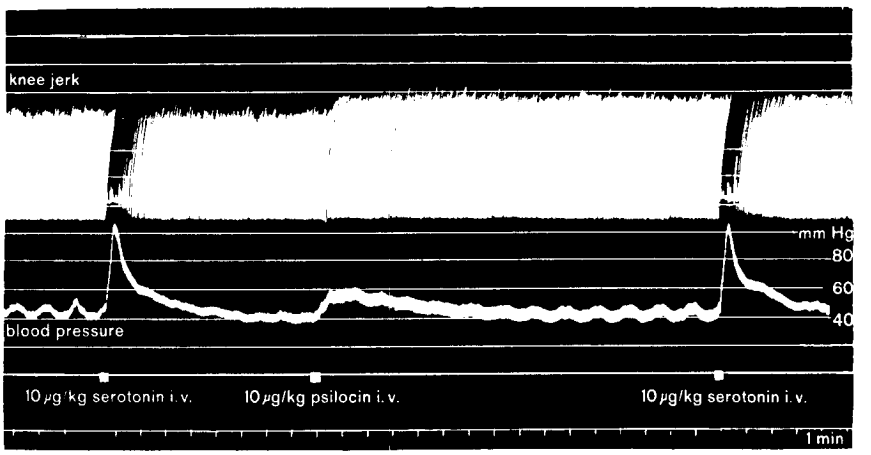


Fig. 1. Effect of psilocin and serotonin on the knee jerk in the spinal cat.

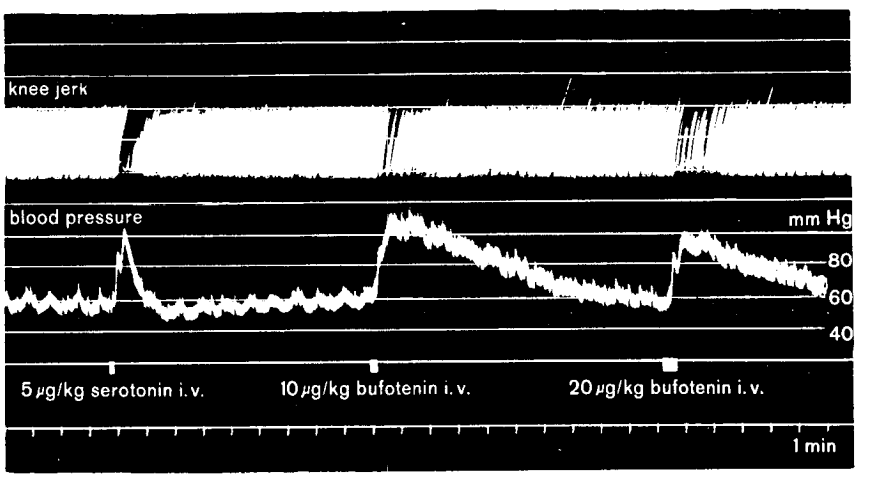
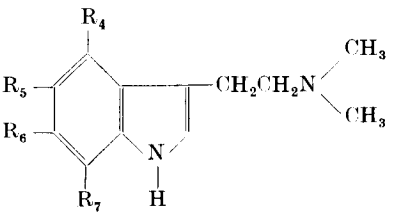


Fig. 2. Effect of bufotenin and serotonin on the knee jerk in the spinal cat.

Results

Since we had observed that 4-hydroxy-dimethyl-tryptamine (psilocin) and its phosphorylated derivative (psilocybin) had just the opposite effect of the two 5-hydroxy-indoles serotonin and bufotenin we tried first to elucidate the importance of the position of the hydroxygroup on the indole ring of several dimethyl-tryptamine derivatives. As well the compounds with a free hydroxygroup were investigated as also the respective derivatives conjugated with phosphoric acid. As shown in table 1 only substances with substitution in the 4- and 5-position show an activity on the patellar reflex,

Table 1*



Substituent	R_4	R_5	R_6	R_7
—OH	↑5–10 (psilocin)	↓20–50 (bufotenin)	no effect	no effect
$—OH_2PO_3$	↑5–10 (psilocybin)	↓ > 50	no effect	no effect

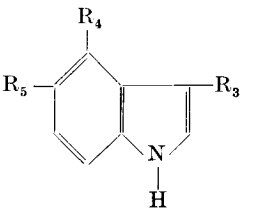
*In this and in the subsequent tables the upward flash means augmentation of the reflex response and the downward flash reflex inhibition. The numbers indicate the minimal i.v. doses in micrograms per kg, which were necessary on the average of experiments to produce alteration of the knee jerk.

whereas 6- and 7-substituted compounds are without any effect up to a dose of 200 μg/kg. With both 4-substituted substances a stimulant effect on the knee jerk is observed which becomes visible already after doses of 5–10 μg/kg and which has a rather long duration (fig. 1). Phosphorylation does not modify the action, neither in the qualitative nor in the quantitative sense. In contrast to this effect a clear cut short-lasting inhibition of the patellar reflex is produced by 5-hydroxy-dimethyl-tryptamine (bufotenin), as illustrated in fig. 2. This effect is identical with that obtained after serotonin, with the only difference that 4 to 5 times higher doses of bufotenin are required. Esterification of bufotenin with phosphoric acid diminishes the activity of the compound without changing its quality.

Our further investigations were concerned with the importance of the side-chain structure in position 3 of the indole-molecule for the activity on the knee jerk. For this purpose only 4- and 5-hydroxylated compounds were selected. All derivatives of 5-hydroxy-tryptamine as listed in table 2 lead to a brief blocking effect on the patellar reflex. Only exceptionally this main action is preceeded by a slight transitory excitation. The highest activity is shown by the unmethylated amine serotonin. All changes in the side-chain of serotonin, like dimethylation, α-methylation, introduction of a piperidine-ring etc. are followed by a moderate and in some cases by a very marked activity decrease. With the exception of 4-hydroxy-tryptamine itself and of its mono-methylated derivative all other compounds derived from 4-hydroxy-tryptamine produce a more or less pronounced excitatory

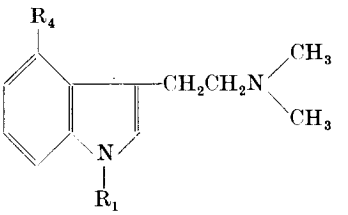
effect on the knee jerk. The most active compound in this series is 4-hydroxy-dimethyl-tryptamine or psilocin. In comparison to this coumpound all alterations in the psilocin side-chain are followed by a decrease or even a complete loss of activity.

Table 2



R_3	R_4 = OH	R_5 = OH
$CH_2CH_2NH_2$	↓20–50	↓5–10 (serotonin)
$CH_2CH_2N(CH_3)H$	↓20–50	–
$CH_2CH_2N(CH_3)_2$	↑ 5–10 (psilocin)	↓20–50 (bufotenin)
$CH_2CH_2N(C_2H_5)H$	↑20–50	–
$CH_2CH_2N(C_2H_5)_2$	↑20–50	–
CH_2CH_2N 	↑20–50	↓> 50
$CH_2CH(CH_3)-NH_2$	↑>50	↓10–20
$CH_2CH(CH_3)-N(CH_3)_2$	↑> 50	↓20–50
$CH(CH_3)-CH_2NH_2$	–	↓> 50
$CH(CH_3)-CH_2N(CH_3)_2$	no effect	–
$CH(OH)-CH_2N(CH_3)_2$	↑> 50	–

Table 3



R_1	R_4	Influence on patellar reflex
CH_3	OH_2PO_3	no effect
CH_3	OH	↑20–50
$CH_2C_6H_5$	OH	no effect

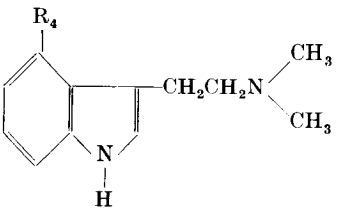
In table 3 we have summarized results, obtained with substances, substituted in position 1, i.e., on the indole-nitrogen of indole. This kind of substitution has proved to be quite active in enhancing the antiserotonin potency of some psilocin-derivatives. Concerning the effect on the knee jerk the 1-methyl-substitution leads to a marked decrease in activity, whereas the 1-benzyl-substituted compound is without any activity.

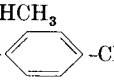
From all the results reported up to now it becomes evident that the stimulatory effect of psilocybin and psilocin on the patellar reflex is a property observed only with derivatives of 4-hydroxy-indoles. It remained to be investigated how far other types of substituents in position 4 would influence this characteristic activity. As illustrated in table 4, a similar effect as with 4-hydroxy- or 4-phosphoryloxy-substitution can also be obtained with other substituents. In the case of the 4-methyl- and 4-methoxy-derivative occasionally some inhibitory effects have occured. From a quantitative point of view the replacement of the hydroxygroup in position 4 by brom or a methyl-, methoxy-, benzyloxy-rest etc. diminishes the activity in most instances quite markedly. The full effect as observed with psilocybin is only present in the case of the benzoic acid ester. If 4-hydroxy-dimethyl-tryptamine is, however, esterified with sulfuric acid, the activity is lost completely, as is the case with a 4-tosylate substitution.

Discussion

The typical enhancing effect on the patellar reflex of the two naturally occuring 4-hydroxyindole-derivatives psilocin and psilocybin differentiates these compounds sharply from the two analogues with the hydroxy- or phosphoryloxy-group in position 5 (bufotenin and 5-phosphoryloxydimethyltryptamine) and from 5-hydroxytryptamine, which all three have a blocking effect on the knee jerk. Within a relatively large series of differently sub-

Table 4



R_4	Influence on patellar reflex
Br	↑ > 50
CH_3	↑ 20–50*
OCH_3	↑ 20–50*
OSO_3H	no effect
$OCH_2C_6H_5$	↑ 20–50
$OCOC_6H_5$	↑ 5–10
$OCONHCH_3$	↑ > 50
OSO_2–  –CH_3	no effect

* Sporadically ↓.

stituted derivatives of tryptamine, the reflex stimulating effect was limited to representatives with a substituent in position 4. Besides this condition also the side-chain configuration is a determining factor, since 4-hydroxytryptamine and its N-mono-methylated derivative still show a serotonin- or bufotenin-like effect, which, however, is reversed to the contrary as soon as the side-chain nitrogen is dimethylated. This relative importance of the tertiary amide-configuration is also illustrated by the fact that the simple dimethyltryptamine without substitution on the indole ring has an effect on the patellar reflex which is qualitatively similar to psilocin, psilocybin and the other 4-substituted substances. However, as soon as a 5-hydroxygroup is introduced in the dimethyltryptamine molecule, the situation changes fundamentally: like 5-hydroxytryptamine (serotonin) also its dimethyl-derivative (bufotenin) shows an inhibitory effect on the knee jerk. Phosphorylation of bufotenin, leading to a compound, which is an isomer of psilocybin, diminishes this blocking effect, but without altering its qualitative aspect. Also in the several other examples of derivatives of 5-hydroxyindole only reflex inhibition was encountered.

It could be tempting to speculate whether the pronounced activation of spinal reflexes by small doses of 4-hydroxyindoles is in some sort connected with the psychotropic activity exerted by these compounds in man. This would naturally not mean to postulate any identity of the two effects, but

rather to suppose that a mechanism of action similar to the one leading to reflex facilitation could also be working at higher levels of the central nervous system. In favour of such an idea is the fact that the psychic syndrome produced by psilocybin in man is also accompanied by an increased sensitivity of the knee jerk [5]. This is also the case in human experiments with lysergic-acid-diethylamide (Delysid®, LSD), and much weight could therefore be added to this hypothesis especially since also LSD shows in the pharmacological test the same effect on spinal reflexes as psilocybin [8, 9]. It must, however, be stressed that in this kind of animal experiment psilocybin and LSD are active at the same dose level, whereas the human dose range for psychotropic effects differs very much, LSD being at least 100 times more potent than psilocybin [5].

Our experiments do not allow a precise indication about the site of action of the facilitatory or inhibitory effect of indole-derivatives on spinal reflexes. An influence of the indole-derivatives on neuromuscular transmission has been excluded by respective experiments [10, 11]. Also the participation of cardiovascular effects in producing the observed alteration of the knee jerk is most unlikely, since all substances tested lead only to some blood pressure rise in the spinal cat, independently whether they enhance or inhibit the patellar reflex. In the case of psilocybin the smallest doses with a clear effect on the knee jerk have sometimes practically no pressor action and in higher doses the moderate pressure rise is very brief in comparison with the relatively long lasting effect on the reflex-activity. Serotonin has on the spinal cat a several times higher pressor activity than psilocybin, but influences the knee jerk in the opposite sense. As shown by *Kissel* and *Domino* [11], also in this case the two actions are independent one from the other, since the blocking effect of serotonin on the patellar reflex persists if the blood pressure reaction is excluded by using a pressure stabilizing system. For further elucidation of the site of action of the influence exerted by the different hydroxyindoles on the patellar reflex it will be necessary to analyze by electrophysiological methods both the afferent limb of the reflex arc and the transmission processes in the spinal cord.

Summary

The 4-hydroxyindole-derivatives psilocybin and psilocin show a characteristic stimulatory effect on the patellar reflex of spinal cats. This is in contrast to the action of the 5-hydroxyindole-derivatives bufotenin and serotonin, which temporarily block the patellar reflex. Using this criterion, a study on structure/activity relationship within a group of about 30 similar indoles was carried out, showing that stimulation of the knee jerk is limited to 4-substituted derivatives of dimethyltryptamine.

Zusammenfassung

Psilocin bzw. dessen phosphorylierte Form Psilocybin, die Wirkstoffe verschiedener hallucinogener mexikanischer Pilze, sind die ersten natürlich vorkommenden Vertreter eines in Stellung 4 substituierten Indolderivates, des Dimethyltryptamins. Bei der pharmakologischen Durchprüfung der beiden Stoffe erwies sich der Patellarsehnenreflex nicht nur als hochempfindliches Kriterium, sondern zeigte sich auch zur Wirkungsdifferenzierung verschiedenartig substituierter Oxyindole geeignet. Während nämlich für Psilocin und Psilocybin eine erregende Wirkung auf den Patellarsehnenreflex der Spinalkatze typisch ist, rufen das dem Psilocin Isomere Bufotenin (5-Oxy-dimethyl-tryptamin) sowie das entsprechende unmethylierte Serotonin (5-Oxy-tryptamin) eine Reflexhemmung hervor. Dieser Befund diente als Ausgangspunkt für die vorliegende Untersuchung, welche die Struktur/Wirkungs-Beziehung innerhalb der Gruppe einfacher Indolabkömmlinge in Form einer Übersicht darstellt.

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