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THE ENZYMIC DEPHOSPHORYLATION AND OXIDATION OF PSILOCYBIN AND PSCILOCIN BY MAMMALIAN TISSUE HOMOGENATES*

A. HORITA and L. J. WEBER

Department of Pharmacology, School of Medicine, University of Washington
Seattle 5, Washington

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Abstract—Incubation of psilocybin with rat kidney homogenates caused a rapid liberation of its —OH congener, psilocin, through the action of alkaline phosphatase. The psilocin thus formed underwent further degradation to form a blue-colored product. This last step appeared to be catalyzed by an oxidative enzyme which was cyanide-sensitive, but not to β-phenyl*iso*propylhydrazine, a monoamine oxidase inhibitor; its optimal activity was at pH 9·0. In many respects it resembles the characteristics of a phenolase type enzyme. The distribution of the phosphatase and oxidase enzymes in a number of tissues from several species of animals was also investigated. Psilocybin-dephosphorylating activity was highest in the rat and mouse kidney and the mucosa of the small intestine of guinea pig and rabbit. Oxidase activity was highest in the heart of all species and in the kidney of the rat and mouse. These experiments indicate that possibly in the intact animal psilocybin is rapidly dephosphorylated and is pharmacologically active as psilocin, while the duration of the effect might be controlled by the oxidation of the latter compound to an *o*-quinone type of structure.

COMPOUNDS possessing indole structures have gained much interest in recent years because of their hallucinogenic actions in man. A considerable number of such compounds are now known and, among these, two stand out because of their unique chemical structures. These are the 4-substituted indoleamines, psilocybin (4-phosphoryl-N: N-dimethyltryptamine) and psilocin (4-hydroxy-N: N-dimethyltryptamine).† Both indoles were first isolated from the mushroom, *Psilocybe mexicana* Heim, and found to be the active hallucinogenic material in this species. The chemistry[1, 2] and various pharmacologic actions[3–5] of these indoles have been described, but little is known as to their metabolism by mammalian tissue systems.

Recent investigations in this laboratory have demonstrated that psilocybin acts as an excellent substrate for the purified alkaline phosphatase enzyme.[6] The dephosphorylation resulted in the liberation of its 4-OH congener, psilocin. The fact that non-specific monophosphoesterases are abundant in various mammalian tissues[7] suggested that possibly a biotransformation of psilocybin to psilocin might occur *in vivo*. This also implied that possibly the active pharmacologic agent after psilocybin administration could be its dephosphorylated congener. The present studies are concerned in part with the *in vitro* dephosphorylation of psilocybin by mammalian

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tissue homogenates. In the course of these studies it was found that not only was psilocybin rapidly dephosphorylated to liberate psilocin, but also the latter compound appeared to undergo further transformation to a blue-colored product. Since the same results were not seen when purified alkaline phosphatase was employed as the enzyme source, it appeared that a second enzyme system was involved. It is the purpose of this report, therefore, to describe some of the characteristics of these two steps in the biotransformation of psilocybin. After some preliminary work it appeared that a dilute kidney homogenate would best serve as the source of the enzymes. The distribution of the phosphatase and the oxidase enzymes was also examined in several species of animals. This was done in order to determine which tissues and species possessed the greatest ability to dephosphorylate and oxidize the 4-substituted indoles. It was considered that by determining their distributions the identification of the oxidase might be aided, especially if it should possess characteristics and distribution patterns similar to those of other known enzymes.

METHODS

Kidneys from male Sprague–Dawley rats were used to prepare the homogenates. The tissue was ground with a Teflon homogenizer in a concentration of 5 per cent with distilled water. All procedures were carried out under ice-cold conditions. With the exception of studies on the effect of pH, a veronal buffer (0·1 M) was employed as the buffer system of the phosphatase and oxidase enzymes, For studies of the pH optimum, a buffer comprised of the following agents was used: Na_2HPO_4 (0·1 M), Tris buffer (0·3 M), β-alanine (0·4 M), and DL-lysine (0·4 M), and adjusted to the proper pH level by the addition of 1·0 N NaOH. This mixture was satisfactory as a buffer in the range of pH from 7 to 10, although it did exert some inhibitory effect on the oxidase system.

The incubation mixture for the determination of phosphatase activity contained the following: 1 ml of kidney homogenate, 1 ml of veronal buffer (0·1 M), 0·3 ml of KCN (0·01 M), and 1 ml of a solution of psilocybin (2 μmoles/ml) in distilled water. The flasks were incubated at 37 °C in a constant temperature Dubnoff-type shaker for the designated times, removed and heated in a boiling water bath for from 3 to 4 min to stop the reaction. Two milliliters of the mixture were then transferred to a shaking bottle containing 15 ml of *n*-butanol, 1 ml of borate buffer (pH 10), and 2 g of solid NaCl. The remainder of the procedure was similar to the method for the colorimetric analysis of serotonin described by Udenfriend *et al.*[8] The final colors of the psilocin were determined spectrophotometically at 430 mμ, as described earlier by Horita and Weber.[6] Known amounts of psilocin were carried throughout the entire extraction procedure and used as standards. As indicated previously, a linear relationship between optical density and concentration was found between the range of from 0·02 to 1·0 μmoles of psilocin per ml.

The oxidase activity was measured both by the disappearance of psilocin and grossly by observation of the formation of a blue color in the incubation mixture. Flasks were arranged similarly as in the phosphatase study and consisted of the following: 1 ml of kidney homogenate, 1 ml of veronal buffer, and 1 ml of a solution of psilocin (from 2 to 4 μmoles/ml). The final pH of the mixture was 8·8. The substrate was added to the flask after equilibration to 37 °C. Two milliliters of the incubation mixture were withdrawn after the appropriate time and transferred to the shaking bottles containing

the *n*-butanol, borate buffer and solid NaCl. The remainder of the procedure for the determination of psilocin is as described above. In determining the species variation of the two enzymes involved, the rat, mouse, guinea pig and rabbit were used as the experimental animals. Brain, liver, kidney, heart and intestinal mucosa were compared for their abilities (1) to dephosphorylate psilocybin and (2) to oxidize psilocin to the blue-colored product. These processes were measured by psilocin liberation from psilocybin and disappearance of psilocin, respectively. Incubations were made as described above, using the 5 per cent homogenate, 2 μmoles of the substrate; the vessels were shaken for a period of 20 min. The remainder of the extraction and analysis was identical to that described above.

RESULTS

The dephosphorylation of psilocybin by a 5 per cent rat kidney homogenate proceeds at an extremely rapid rate. As can be seen in Fig. 1, an essentially maximal

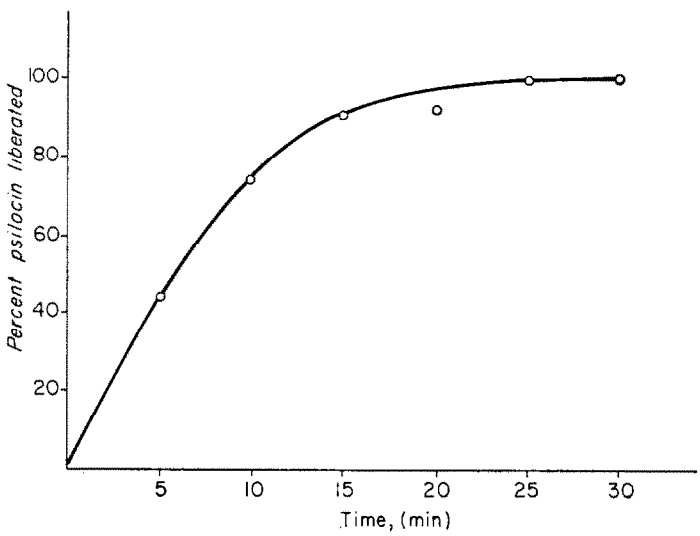

FIG. 1. The rate of psilocin liberation from psilocybin by rat kidney homogenate. Psilocybin (2 μmoles) was incubated with 1 ml of kidney homogenate (5 per cent), 10^{-3} M KCN, and 0·1 M veronal buffer. Final volume, 3·3 ml; pH 8·8. Temperature 37 °C.

liberation of psilocin was accomplished within 15 min of incubation, indicating that the alkaline phosphatase activity in kidney tissue was relatively high. The effect of altering pH on the dephosphorylation of psilocybin was also investigated. Fig. 2 shows a single peak of pH 9·0, indicating the optimal pH for the activity of alkaline phosphatase. Even at the physiological pH of 7·4 a considerable amount of dephosphorylation was evident.

In all of the experiments with the phosphatase enzyme, KCN was added to the incubation mixture (final concentration, 10^{-3} M). In the absence of KCN the total amount of psilocin formation was not measurable, because of its rapid degradation by a cyanide-sensitive enzyme system which will be described below. KCN at 10^{-3} M

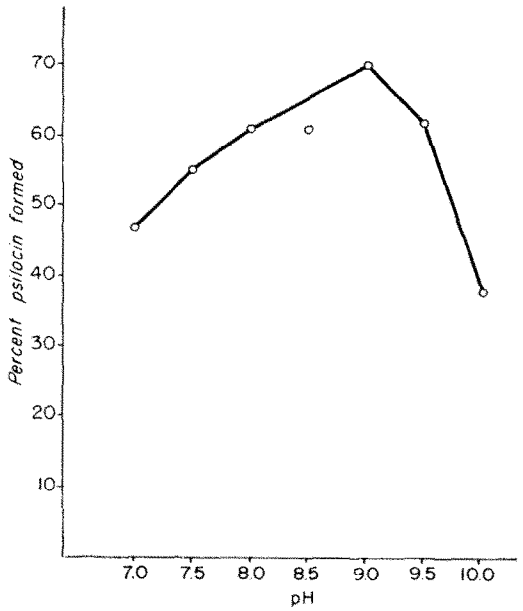

FIG. 2. The effect of pH on psilocin liberation from psilocybin by rat kidney homogenate. Psilocybin (2 μmoles) was incubated with 1 ml of kidney homogenate (5 per cent), 10^{-3} M KCN, and buffer (described in text) for 40 min at 37 °C.

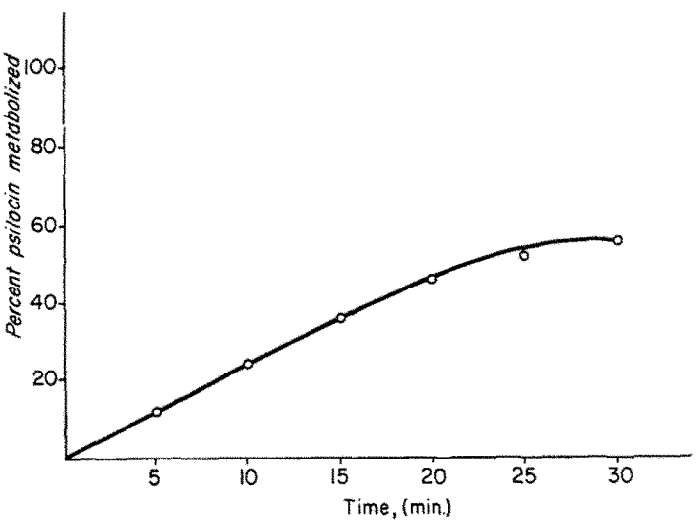

FIG. 3. The rate of disappearance of psilocin in rat kidney homogenate. Psilocin (2 μmoles) was incubated with 1 ml of kidney homogenate (5 per cent) in 0·1 M veronal buffer. Final volume, 3 ml; pH 8·8. Temperature 37 °C.

was completely effective in protecting the psilocin from degradation, but did not influence the phosphatase activity.

Incubation of psilocin with rat kidney homogenate in veronal buffer (final pH, 8·8) resulted in psilocin disappearance at a linear rate over a 25-min period (Fig. 3). Beyond this period, the rate of breakdown decreased indicating that a submaximal

substrate concentration had been reached. The disappearance of the psilocin was accompanied by the development of a blue color in the incubation mixture. When higher concentrations of psilocin and kidney homogenate were used the development of the blue color was extremely rapid and intense, reaching a dark blue within a matter of minutes. The addition of KCN (final concentration 10^{-3} M) completely prevented the appearance of the blue color, as well as the disappearance of psilocin. When the reaction was carried under a nitrogen atmosphere similar results were found as with KCN, indicating that the degradation of psilocin by rat kidney homogenate involved an aerobic oxidative process. The monoamine oxidase inhibitor, β-phenyl*iso*propylhydrazine, exerted no influence on the psilocin breakdown reaction.

The pH optimum of this oxidative reaction was determined. As shown in Fig. 4, the reaction progressed with increased pH until the optimum of pH 9·0 was reached.

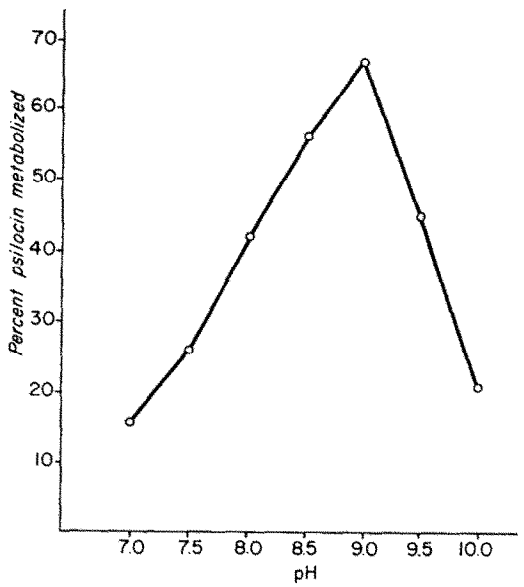

FIG. 4. The effect of pH on the disappearance of psilocin in rat kidney homogenate. Psilocin (2 μmoles) was incubated with 1 ml of kidney homogenate (5 per cent) in buffer (described in text) for 40 min at 37 °C.

Beyond this point the activity fell rapidly. Like the phosphatase reaction, the oxidation of psilocin also occurred to some extent at the pH of 7·4.

The distribution of the phosphatase and oxidase enzymes acting upon psilocybin and psilocin, respectively, in the various species of animals is tabulated in Table 1. All values are expressed as per cent substrate metabolism (psilocybin by phosphatase, psilocin by the oxidase). A noticeable resemblance is apparent in the distribution of the enzymes in the various animals. This is especially true in the mouse and rat, in which case all of the activities in the tissues investigated were quite similar. Kidney phosphatase in these two species were especially high, while in the guinea pig and rabbit the mucosa of the small intestine displayed the highest phosphatase activity. Under the conditions of these experiments these tissues were capable of dephosphorylating essentially the entire amount of psilocybin present. In all animals the liver and heart were low in their ability to dephosphorylate psilocybin.

The extent of oxidation of psilocin also varies with the species and the source of the enzyme. In all of the species the heart exhibited more oxidase activity than any of the other tissues tested. The rat and mouse kidney also showed high activity. The lowest activity was consistently found in the small intestine.

DISCUSSION

That mammalian tissues can dephosphorylate psilocybin with the liberation of psilocin is evident from the present studies. The rate of dephosphrylation, as shown with the 5 per cent rat kidney homogenate, indicates a high activity of the enzyme in this tissue. Since the reaction occurs at pH levels in the physiological range, as well as at the optimum of pH 9, it is most probable that psilocybin dephosphorylation

TABLE 1. ALKALINE PHOSPHATASE AND PSILOCIN-OXIDIZING ACTIVITIES OF VARIOUS TISSUE HOMOGENATES FROM FOUR SPECIES OF ANIMALS

Figures are expressed as per cent substrate metabolism (psilocybin by phosphatase, psilocin by oxidase). Figures represent the mean values of from three to four determinations.

	Rat		Mouse		Guinea pig		Rabbit	
Enzyme / Tissue	Phos-phatase	Oxidase	Phos-phatase	Oxidase	Phos-phatase	Oxidase	Phos-phatase	Oxidase
Kidney	99	57	95	57	65	27	34	39
Heart	14	61	13	65	7	64	4	68
Brain	14	28	28	32	20	25	5	30
Liver	3	35	1	37	3	25	5	8
Small intestine	13	17	10	14	88	15	96	9

occurs *in vivo* as well as *in vitro*. Evidence that this takes place in the intact rat has been observed (unpublished observations).

The rapid transformation of psilocybin to psilocin raises the question as to whether the former compound plays any role in exerting the central nervous system effects which are seen after its administration to either man or animals. It would appear likely that the active substance is its dephosphorylated congener, psilocin, since it possesses greater lipid solubility (unpublished observations) and would theoretically pass the blood–brain barrier more readily than psilocybin. It would be interesting to determine whether a specific inhibitor of the phosphatase enzymes would delay or inhibit the actions normally exerted by psilocybin.

The oxidation of the psilocin liberated from the psilocybin by the kidney homogenate was an unexpected finding. In the earlier exploratory experiments kidney homogenates at concentrations as high as 33 per cent were employed for the phosphatase reaction. Upon adding the psilocybin to the incubation mixture a blue color developed within a few minutes, and within 10 min turned into an intense blue-black mixture. When psilocin was incubated in the same manner it also disappeared with the formation of the blue color. With other tissue homogenates, such as the heart, psilocybin did not display the blue color as rapidly as with kidney; however, psilocin was quickly converted to the colored state. It appears that in order to be oxidized to the blue-colored product, psilocybin must first be dephosphorylated to psilocin. The nature of

this oxidation product is unknown. Recently, Blaschko and Levine[9] described the presence of a hydroxyindole oxidase in the gill plates of *Mytilus edulis*; this enzyme was capable of oxidizing psilocin with the formation of a blue-colored product. The authors suggested that the final product might be of an *o*-quinoid structure. It is probable that the oxidation of psilocin by the *Mytilus* oxidase results in the same product as found with the kidney homogenate, but it is unlikely that the enzymes in the two systems are identical.

The high pH optimum of the rat kidney oxidase system is unusual, and it was first thought that possibly the oxidation of psilocin was a non-enzymatic auto-oxidation similar to that found for DOPA at alkaline pH.[10] However, the fact that a pH optimum peak was present indicated that the reaction was enzymic. No auto-oxidation of psilocin was found when the compound was incubated with veronal buffer at pH 9·0 for the times designated in the section on Results. Incubation with kidney homogenate buffered at pH 11 or above showed almost no breakdown of psilocin. Furthermore, definite differences in activity were found among the various tissues and in the different species findings which suggest that this was not a non-specific auto-oxidation process. Finally, exposing the homogenate to a boiling water bath abolished its enzymic properties.

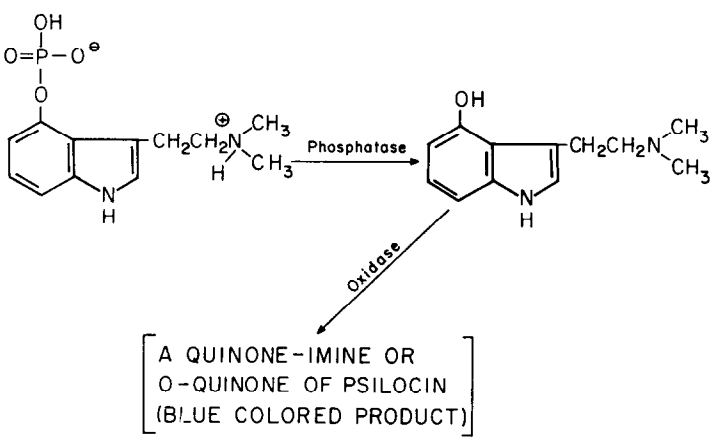

FIG. 5. Diagram representing the metabolic pathway of psilocybin. The structures in brackets indicate hypothetical oxidation products of psilocin.

Preliminary work with possible inhibitors of this oxidase system establishes the fact that monoamine oxidase is not involved. Not only do the optimum conditions differ, but also the potent monoamine oxidase inhibitor, β-phenyl*iso*propylhydrazine, has no influence on the oxidative process. KCN, on the other hand, which does not block monoamine oxidase, was effective in inhibiting the degradation of psilocin. The distribution of the oxidase activity in the various tissues also does not resemble that of monamine oxidase. Generally, the liver is the richest source of monoamine oxidase, and heart and kidney are relatively low; however, the oxidase which is active against psilocin is highest in the heart and kidney, with the liver showing only minimal activity.

Whatever the nature of this oxidative process might be it is evident that homogenates prepared from tissues of various animals are capable of dephosphorylating psilocybin to form psilocin, and the latter further metabolized to form a blue-colored product. In the rat kidney the oxidative process appears to be the rate-limiting step, since it was shown that under the conditions employed, dephosphorylation proceeded at a much faster rate than did the disappearance of psilocin. This does not hold true for all tissues, however, since the findings with preparations like heart homogenate, which possess low phosphatase activity, indicate that this enzyme catalyzes the rate-limiting step. The scheme, Fig. 5, illustrates diagrammatically the pathway of degradation of psilocybin. The structures in brackets are hypothetical, but since the phenolase reaction involves the hydroxylation of the *o*-position of the ring, followed by an *o*-quinone formation,[11] we suggest, as did Blaschko and Levine,[9] that the *o*-quinone of psilocin is responsible for the blue color. Our recent studies with a rabbit heart mitochondria preparation and a purified pig heart cytochrome oxidase indicate that a phenolase-like action of this enzyme may be involved in this oxidation process.

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