Dephosphorylation of Psilocybin in the Intact Mouse[1,2]

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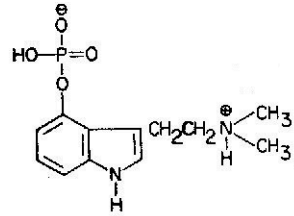
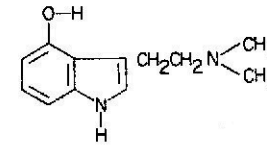
Several of the indoleamines found in the mushroom *Psilocybe mexicana* Heim have been identified as being 4-substituted compounds (Hofman *et al.*, 1958, 1959). These are psilocybin (4-phosphoryl-*N*,*N*-dimethyltryptamine) and its 4-hydroxy analog, psilocin, the structures of which are shown below. Both compounds possess hallucinogenic activity in man and produce central nervous system effects in experimental animals.



Psilocybin



Psilocin

Thus far, only a few studies have been reported on the metabolism of these compounds (Erspamer *et al.*, 1960; Gessner *et al.*, 1960; Blaschko and Levine, 1960a,b). In some of our earlier work we showed that the phosphoryl group of psilocybin could be removed by incubation with purified alkaline phosphatase or with various mammalian tissue homogenates, resulting in the liberation of the 4-hydroxy analog, psilocin (Horita and Weber, 1961a;b). Since this occurred even at physiological levels of pH, it suggested to us that possibly in the intact animal

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psilocybin might also be dephosphorylated and that its central nervous system action might be exerted by the psilocin rather than the phosphorylated compound. This is a reasonable assumption, for in determining the oil-water solubilities of the two compounds at pH 7.4, psilocin was found to be much more soluble in organic solvents than was psilocybin. Since lipid solubility is considered to be an important property for a compound to pass the blood-brain barrier it may be assumed that, all else being equal, psilocin would be more effective in entering the brain to produce its pharmacologic actions. Furthermore, it is a well established fact that various mammalian tissues possess an abundance of phosphatases which could possibly be responsible for the dephosphorylation process.

The present study, therefore, was undertaken to determine whether psilocybin could be dephosphorylated *in vivo* and whether the central nervous system activity of this agent was produced by its 4-hydroxy congener.

METHODS

All experiments were performed on male albino mice weighing 25–35 g. Drugs were injected intraperitoneally, and psilocybin and psilocin were administered in amounts of 100 mg/kg and 72 mg/kg, respectively, which represent equimolar (0.35 mmole/kg) doses. In those instances in which sodium β-glycerophosphate (45 mg per mouse) was employed as a competitive substrate, the psilocybin or psilocin was administered 5 minutes after pretreatment. After administration of these agents the mice were sacrificed at 5-minute intervals and the brain, liver, and kidney were removed for analysis of psilocin contents. In all cases the organs of two animals were pooled in order to provide adequate quantities of tissue. All tissues were then homogenized in 0.1 *N* HCl with a Teflon tissue grinder.

The homogenates were carried through the extraction and analytical procedure as described for the colorimetric determination of 5-hydroxytryptamine (Udenfriend *et al.*, 1958). However, larger volumes of homogenates were employed so the measurable amounts of psilocin could be recovered. The final spectrophotometric readings were made at 365 mμ and/or at 430 mμ. Tissue blanks and standards were also carried through the entire procedure.

Behavioral changes in psilocin- and psilocybin-treated animals were found by direct observation. The descriptions of these observations are found in the section under Results.

RESULTS

The intraperitoneal administration of 100 mg/kg of psilocybin to mice resulted in the accumulation of measurable amounts of psilocin in liver and kidney tissue within 5 minutes. Peak levels were reached in about 10–20 minutes, after which they gradually declined. Kidney tissue demonstrated the highest accumulation of psilocin of the three organs studied, reaching concentrations of 120 μg per gram of tissue. Liver

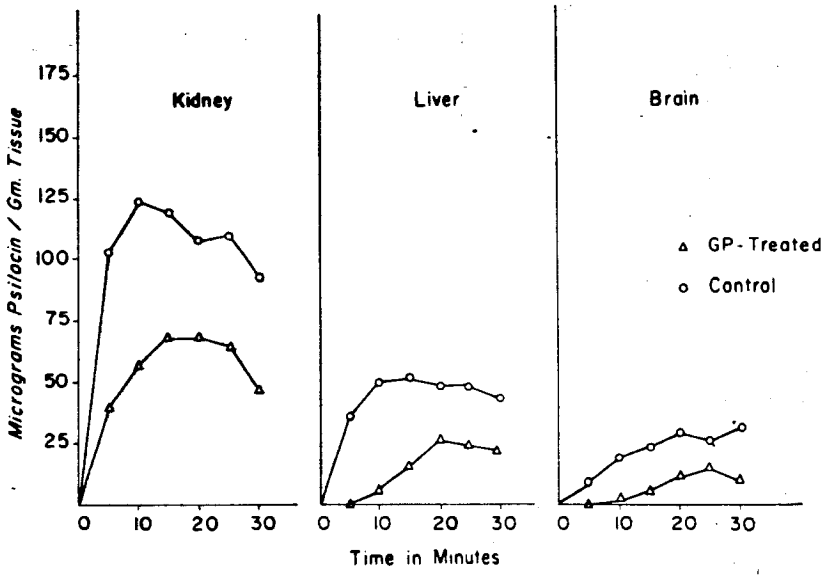

FIG. 1. Graph representing the concentrations of psilocin in the kidney, liver, and brain in control and sodium β-glycerophosphate (GP) pretreated rats after intraperitoneal administration of 100 mg/kg of psilocybin. Each point represents the mean values of three determinations in which 6 mice were employed.

showed about one-half this amount. The brain did not attain levels of psilocin as high as the other organs, and the peak of its concentration curve was delayed until 20–30 minutes after administration. The results of psilocin accumulation after psilocybin administration are shown in Fig. 1.

Observations of the behavior of the animals treated with psilocybin also corresponded well with the time of maximum brain concentrations of psilocin. The major symptoms consisted of piloerection, exophthalmos, some hindleg ataxia, and depression. In all instances the effects were

completely reversible, and with the exception of some depression most animals appeared normal within 3–4 hours after psilocybin administration. Because of the difficulty in accurate measurement of such responses, no attempt was made to evaluate the observations quantitatively. The observations were made by several unbiased observers, and the decisions were generally in agreement.

In those animals pretreated with 45 mg of sodium β-glycerophosphate per mouse 5 minutes prior to psilocybin, the accumulation of psilocin

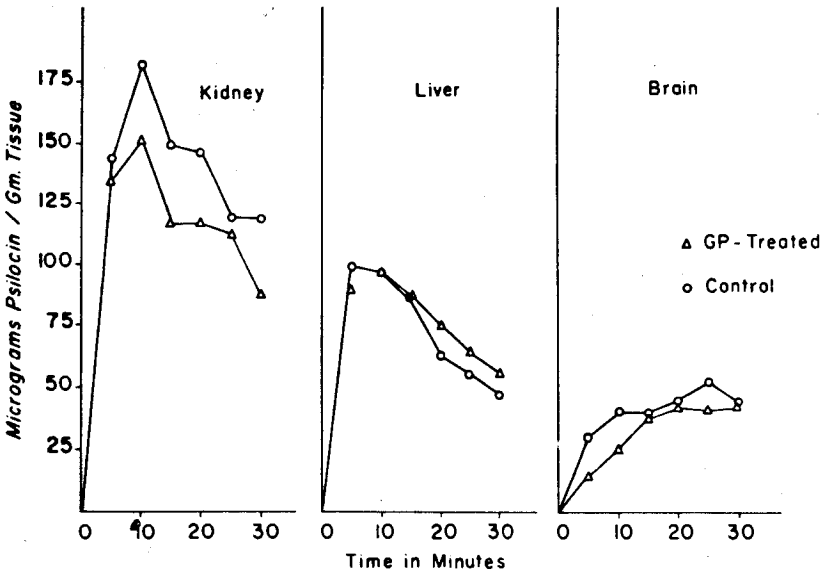

FIG. 2. Graph representing the concentration of psilocin in the kidney, liver, and brain in control and sodium β-glycerophosphate (GP) pretreated rats after intraperitoneal administration of 72 mg/kg of psilocin. Each point represents the mean values of three determinations in which 6 mice were employed.

in all tissues was reduced markedly. These results are shown in Fig. 1. The peaks of their concentration curves were also shifted so that the highest amounts were found at least 15 minutes after psilocybin injection. In kidney and liver tissues the psilocin concentrations were below 70 μg/g and 30 μg/g, respectively, at their peak levels. Psilocin in brain was not measurable to any extent during the first 10–15 minutes; after this a gradual increase was noted. This amount, however, was less than half of that found in mice given psilocybin alone.

The dose of β-glycerophosphate employed in these studies did not produce any observable alterations in behavior. The administration of psilocybin to such animals, however, delayed the onset and reduced the intensity of the reaction as compared to normal mice treated with psilocybin.

The tissue concentrations of psilocin after its administration were considerably higher than after psilocybin. Kidney tissue exhibited levels as high as 180 μg/g within 10 minutes after injection. In general, all tissues reached higher levels and at more rapid rates than from mice treated with psilocybin. In some instances, the levels of psilocin in the kidney fluctuated widely. A major difference between the tissue concentrations of psilocin in psilocybin- and psilocin-treated animals was the pattern displayed in the liver. With the former treatment psilocin levels rose gradually and tended to maintain a plateau, whereas in the latter treatment the levels rose very quickly, but upon reaching a peak, rapidly declined. At the end of the 30 minutes, however, the livers of both the psilocybin- and psilocin-treated animals showed about the same values of psilocin concentrations. Brain levels of psilocin in psilocin-treated mice followed the relatively slow onset and peak as seen in the psilocybin-treated animals; their final tissue concentrations, however, were considerably higher. These results are shown in Fig 2.

When psilocin was given to mice pretreated with sodium β-glycerophosphate, the tissue concentrations were essentially the same as those in normal animals with psilocin. Levels rose at about the same rates and to about the same extent over the 30-minute period. The apparent difference between the kidneys from the β-glycerophosphate-pretreated and normal mice was not statistically significant. The behavioral changes of mice given psilocin, whether untreated or pretreated with β-glycerophosphate, could not be differentiated. The onset, intensity, and duration of action appeared to be identical in the two groups.

DISCUSSION

The purpose of the present study was twofold. First, it was to determine whether psilocybin could be dephosphorylated to liberate psilocin in the intact animal and under physiological conditions. The second purpose was to determine whether the central nervous system actions of psilocybin might be produced by the psilocin which was liberated by the enzymatic process.

That dephosphorylation of psilocybin and the subsequent liberation

of psilocin can occur in the intact mouse has been firmly demonstrated in the present study. This reaction is extremely rapid, for within 5 minutes after the intraperitoneal injection of psilocybin, large amounts of psilocin can be found in the kidney and liver. The conversion of psilocybin to psilocin *in vivo* is probably catalyzed by tissue and serum alkaline phosphatase, for psilocybin is an excellent substrate for this enzyme *in vitro*. The extent to which psilocin was found in the tissues was approximately proportional to the degree of alkaline phosphatase present in that tissue. Thus, in our earlier studies on the dephosphorylation of psilocybin by mammalian tissue homogenates, we had found mouse kidney to be extremely active whereas liver was very low in activity. Brain alkaline phosphatase activity is generally higher than that of liver, but in the present results brain concentration of psilocin after psilocybin administration was the lowest of the three organs studied. This may be expected, however, because psilocybin, due to its low lipid solubility, probably does not pass the blood-brain barrier. Upon dephosphorylation to psilocin, however, it becomes highly lipid soluble, and in this form may enter the brain. It therefore appears that the central actions of psilocybin are exerted only after its conversion to psilocin. This is further supported by the fact that in the presence of a competitive substrate, sodium β-glycerophosphate, the amount of psilocin found in the brain was much lower than in untreated brains, and this corresponded well with the intensity of the behavioral actions. The attenuated response to psilocybin in the β-glycerophosphate-pretreated mice was not caused by an antagonistic effect, for when psilocin was used, both pretreated and untreated animals responded similarly.

Sodium β-glycerophosphate was selected as a competitive substrate in order to reduce the amount of psilocybin dephosphorylation. A competitive inhibitor which would be effective *in vivo* would have been a more desirable choice in this case, but thus far we have not found such a compound. In order to produce the desired degree of enzyme competition, a relatively large amount of the glycerophosphate was required. This dose, however, did not produce any observable behavioral effects, nor did it interfere with the actions of injected psilocin. Because of its low toxicity as well as that of its dephosphorylated product, glycerol, it has served as a satisfactory agent to inhibit the dephosphorylation of psilocybin.

These results therefore demonstrate the *in vivo* dephosphorylation of psilocybin and also strongly suggest that in the mouse the centrally active agent is the dephosphorylated form. Whether this is the case in other

animals, or whether it is involved in the peripheral pharmacologic actions of psilocybin, is not known. The extremely rapid rate of dephosphorylation as seen in these experiments suggests that at least part of the peripheral response to psilocybin also may be exerted by psilocin.

SUMMARY

The administration of psilocybin (4-phosphoryl-*N*,*N*-dimethyltryptamine) to mice resulted in the accumulation of its 4-hydroxy analog, psilocin, in the kidney, liver, and brain. Highest concentrations in kidney and liver were found within 10–20 minutes and brain levels reached a peak at 25–30 minutes after administration of psilocybin. Behavioral effects, characterized by piloerection, exophthalmos, and motor incoordination, closely followed the increase in brain levels of psilocin. In mice pretreated with large doses of sodium β-glycerophosphate, a substrate of alkaline phosphatase, the administration of psilocybin resulted in much lower increases in tissue psilocin levels as compared to controls, and the behavioral effects were correspondingly attenuated.

The accumulation of tissue psilocin after administration of psilocin did not differ significantly in control or β-glycerophosphate-pretreated mice. Correspondingly, no difference in the intensity of the behavioral effects could be observed in control and glycerophosphate-pretreated mice.

The results of this study demonstrate the rapid dephosphorylation of psilocybin by the intact mouse. Evidence is also presented that the central nervous system effects of psilocybin are exerted only after its transformation to the 4-hydroxy analog, psilocin.

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