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PSYCHOTOMIMETIC DRUGS AND BRAIN 5-HYDROXYTRYPTAMINE METABOLISM*

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Abstract—The effects of indolealkylamine and phenylethylamine psychotomimetic drugs on brain 5-hydroxytryptamine (5-HT) metabolism have been studied. Indolealkylamines (psilocybin, psilocin, *N,N*-dimethyltryptamine or DMT) have a lysergic acid diethylamide (LSD)-like effect (increased 5-HT with concomitant decrease in 5-hydroxyindoleacetic acid, 5-HIAA). A degree of inhibition of monoamine oxidase can be demonstrated with psilocybin and psilocin, but not with LSD. The data suggest that other factors are operative in producing decreased levels of 5-HIAA. Phenylethylamines (mescaline, 2,5-dimethoxy-α,4-dimethylphenylethylamine or DOM) not only increase 5-HT but also, at higher doses, 5-HIAA; at lower doses, there is some indication of an LSD-like effect on 5-HIAA. Both categories of drug influence 5-HT metabolism, and the noted differences with respect to 5-HIAA cannot as yet be accounted for.

SPECULATION that *d*-lysergic acid diethylamide (LSD) produced effects of interfering with the action of serotonin (5-HT) in brain was based on structural resemblances (an indole nucleus), interactions observed in certain smooth muscle preparations, the presence of serotonin in brain and the differential distribution of the decarboxylase in brain:[1] The lack of correlation of peripheral antiserotonin potency with psychotomimetic effect was demonstrated, but it was also shown that, particularly at low concentrations, LSD had serotonin-like action in various smooth muscle systems[2–4] or metabolic systems in the liver fluke.[5]

When interaction of drug and amine were studied in brain, significant increases of 50–100 mμg/g of 5-HT were noted after doses of 130 μg LSD/kg or higher, the increases being confined almost entirely to the high speed particulate fraction of brain homogenates.[6–9] Mescaline, psilocybin and only the psychoactive congeners of LSD were found to produce small but significant increases in whole rat brain levels of 5-HT;[7, 10] *N,N*-dimethyltryptamine or psilocin produced similar effects in rabbit brain.[11, 12] LSD was also found to decrease whole brain levels of norepinephrine[7, 13] and to decrease levels of brain 5-HIAA.[14–17]

Giarman and Pepeu[18] found no 5-HT effects with cholinolytic deliriants which, however, did influence brain levels of acetylcholine; these appear to constitute, on behavioral as well as biochemical grounds, another class of psychotomimetics.[19] While small 5-HT changes appear to characterize the indolealkylamine and phenylethylamine psychotomimetics, no systematic survey of relevant psychotomimetic drugs had been undertaken in terms of their effects on both brain 5-HT and 5-HIAA. The

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present report presents the initial findings of a study investigating the extent to which classes of psychotomimetic drugs (defined either behaviorally or in terms of cross-tolerances, or on the basis of molecular structure) could also be defined in terms of changes in brain metabolism of 5-HT.

METHODS

Design of experiments. Male Sprague–Dawley rats (Madison, Wisconsin), 150–250 g, were used. Experimental rats received drugs by intraperitoneal (i.p.) injection and were sacrificed by decapitation at various time intervals after drug administration. Following decapitation, brains were immediately removed and homogenized in either 4 vol. of 0·1 N HCl (for 5-HT, 5-HIAA assays) or in Ringer phosphate or sucrose (for MAO studies, as described below). Drugs were dissolved in saline, with pH adjustment when necessary. Control animals received saline or drug vehicle. All experimental data were compared with control data obtained in the same experiment. Each individual experiment was designed so that a minimum of 25 per cent of the animals were controls. No more than sixteen animals were studied in any single experiment. Student's *t*-test was used for determining statistical significance.

5-HT and 5-HIAA assays. Both 5-HT and 5-HIAA were estimated in the same brain samples by a modification[16] of methods for 5-HT[20] and 5-HIAA.[21] In separate experiments it was determined that mescaline, 2,5-dimethoxy-α,4-dimethylphenylethylamine (DOM), psilocin, psilocybin, or dimethyltryptamine (DMT) did not interfere with these measurements. These drugs did not show fluorescence peaks at the wavelengths at which 5-HT and 5-HIAA are measured (activation, 295 mμ; emission, 540 mμ) either when directly added to the cuvette, or when carried through the assay procedure, or when added to assay tubes containing tissue samples. There was no increased fluorescence of 5-HT or 5-HIAA with these procedures. Recovery of added 5-HT and 5-HIAA was similarly unchanged in the presence of added drug.

Monoamine oxidase (MAO) studies. The effect of drugs on MAO activity was studied in two ways: first, by assays of MAO activity in whole brain homogenates from drug-treated animals, and secondly, by assays of MAO activity in lysed mitochondrial preparations to which were added different concentrations of drugs.

Brains from drug-treated animals were homogenized at 0–4° within 3 min of death in 9 vol. of Ringer phosphate solution (pH 7·4). After a preincubation of 30 min at 37° in a Dubnoff metabolic shaker, 10 μg 5-HT were added to aliquots of the homogenate containing 250 mg brain tissue. The total volume was 2·6 ml. The mixtures were incubated at 37° and the reactions stopped after 15 min by the addition of 1 ml of 6N HCl. Aliquots (3 ml) of the suspension were withdrawn and assayed for 5-HT by fluorescence spectrometry. The results of this determination of residual 5-HT were confirmed radiometrically by the use of ^{14}C-5-HT as substrate and measurement of the specific activity of the residual 5-HT in the extract of the reaction mixture at the end of the incubation period. Homogenates from saline-injected animals served as controls. The extent of inhibition was determined by comparing the amounts of residual 5-HT in drug-treated and control homogenates. MAO data reported below are the results of five experiments involving fifteen animals.

The lysed mitochondrial preparations were made according to the method of Gray and Whittaker[22] with the following modifications. The crude mitochondrial fraction (P_2) was resuspended in 0·32 M sucrose (5 ml), layered over 5 ml of 1·2 M sucrose, and

centrifuged at 53,500 *g* for 2 hr. Mitochondria in the resulting pellet were lysed by homogenization in 0·05 M phosphate buffer (pH 7·4). The lysed suspensions could be stored at −70° for at least 2 weeks without detectable loss of enzyme activity. MAO activity was estimated by following the disappearance of 5-HT.[23] For this assay, 1 ml of lysed mitochondrial suspension was added to 1·3 ml 0·5 M phosphate buffer (pH 7·4). After a 30-min preincubation in the Dubnoff incubator at 37° in stoppered 25-ml Erlenmeyer flasks, 600 mμg 5-HT and the presumptive inhibitor were added. The total volume was 3·0 ml. The reaction was stopped at 5-min intervals by addition of 1 ml 6 N HCl. Aliquots (3 ml) were assayed for residual 5-HT. Essentially similar results as reported below were obtained when ^{14}C-5-HT was used as a substrate. Graphs of residual 5-HT versus time showed that the rate of destruction of 5-HT was constant for at least the first 15 min of the reaction. The degree of inhibition was estimated by comparing the amount of remaining 5-HT in experimental (drug-treated) flasks with control flasks. The *in vitro* data reported below represent data from six experiments. In each experiment, the mitochondrial suspensions were prepared from four brains.

RESULTS

Effects of psychotomimetic drugs on brain 5-HT and 5-HIAA concentrations. These experiments have investigated the effects of psychotomimetic drugs on 5-HT metabolism in brain. The data show a distinct difference between the hallucinogenic

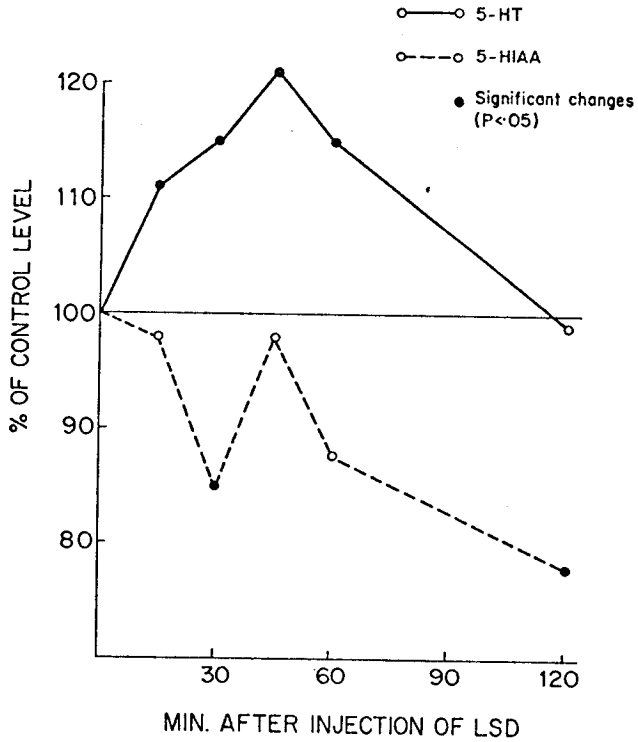

FIG. 1. Time course of the effect of 520 μg/kg *d*-LSD (i.p.) on the metabolism of brain 5-HT. The changes in 5-HT and 5-HIAA are plotted as per cent of the control levels at each time.

phenylethylamines (mescaline, DOM) on the one hand, and indolealkylamines (psilocybin, psilocin, *N*,*N*-dimethyltryptamine, LSD) on the other. Figure 1, redrawn from a previous publication,[16] shows that a dose of 520 μg/kg of *d*-LSD, administered i.p., induced significant changes in both 5-HT and 5-HIAA. Other non-hallucinogenic congeners do not have this effect. In experiments involving sixty-four animals, there were no changes either in 5-HIAA or 5-HT levels 30 min following 520 μg *l*-LSD/kg; 520–1300 μg BOL (2-bromo-*d*-lysergic acid diethylamide)/kg; 520 μg (methysergide)/kg. Less potent LSD-related hallucinogens, such as 1-methyl-*d*-LSD (MLD), show, as

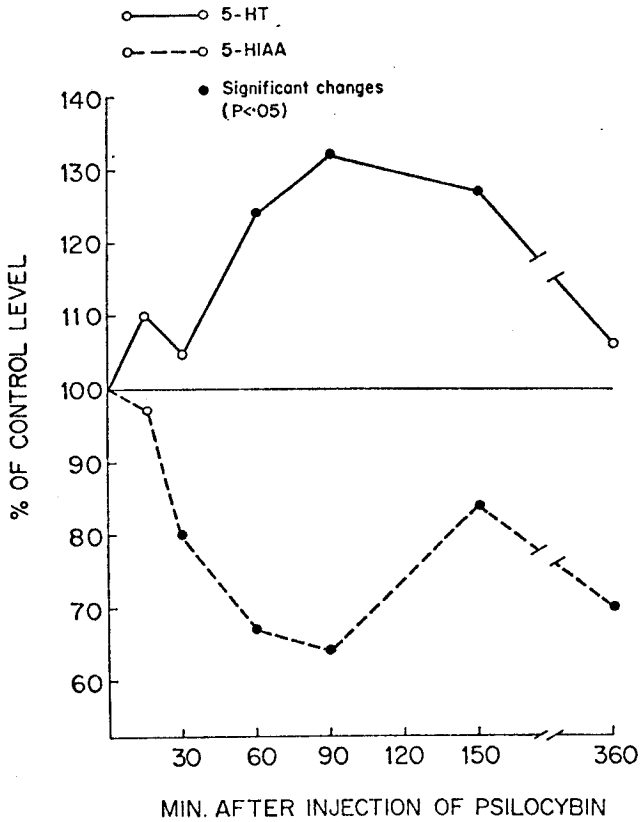

FIG. 2. Time course of the effect of 25 [mg/kg] psilocybin on the metabolism of brain 5-HT. The changes in 5-HT and 5-HIAA are plotted as per cent of the control levels at each time. The same number of animals was used in the control group as in the experimental group: 15 min, 6; 30 min, 8; 60 min, 6; 90 min, 9; 150 min, 6; 360 min, 10.

previously reported, a tendency toward a significant effect on 5-HT metabolism, particularly at higher doses; 30 min after 520 μg/kg there is a 15 per cent decrease in 5-HIAA ($P < 0{\cdot}1$) and after 1300 μg/kg, a 27 per cent decrease ($P < 0{\cdot}01$). Other indoleamine hallucinogens show a similar pattern of effect on brain 5-HT, but of longer duration. The effect of psilocybin, 25 mg/kg, on brain 5-HIAA is maximal at 90 min and is still present at 6 hr (Fig. 2). A higher dose (50 mg/kg) of psilocybin, as well as 25 mg/kg psilocin, gave a comparable effect at 90 min. After 5-methoxy-

tryptamine (20 mg/kg) the brain concentration of acid metabolite measured as 5-HIAA increases markedly (313 per cent of control), but the possibility of interference by 5-methoxyindoleacetic acid remains to be determined. The effects of *N,N*-dimethyltryptamine (DMT) are similar to those of psilocybin but of shorter duration (Fig. 3).

Psilocybin and psilocin appear to have some inhibitory effect on brain MAO *in vivo*. After 15 min incubation of brain homogenates from animals sacrificed 90 min after a dose of 25 mg psilocin or psilocybin/kg, the amount of residual 5-HT was 12 per cent (psilocin, $P < 0{\cdot}001$) or 9 per cent (psilocybin, $P < 0{\cdot}001$) more than in homogenates

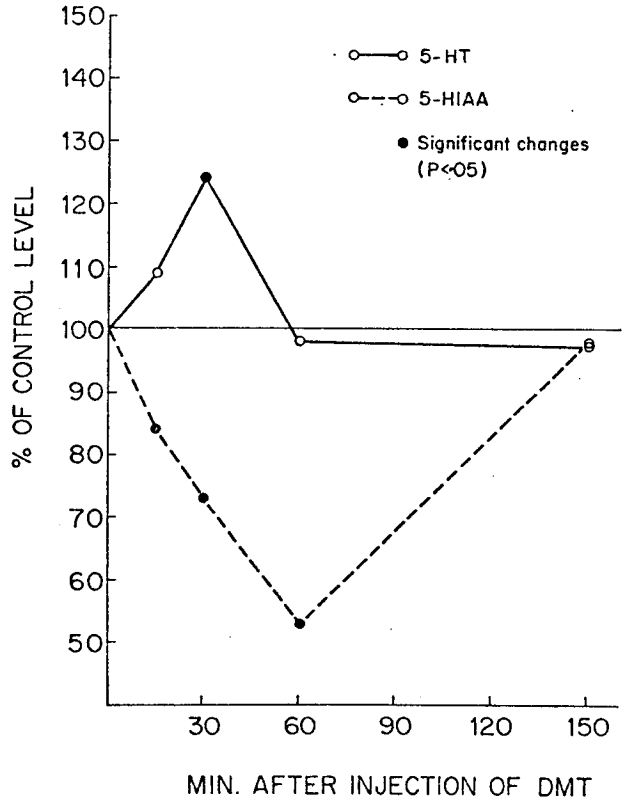

FIG. 3. Time course of the effects of [20 mg/kg] *N,N*-dimethyltryptamine on the metabolism of brain 5-HT. The changes in 5-HT and 5-HIAA are plotted as per cent of the control levels at each time. Control and experimental groups had equal numbers of animals: 15 min, 6; 30 min, 10; 60 min, 4; 150 min, 12.

from the saline-treated controls. There was no such effect in animals injected with 520 μg LSD/kg and sacrificed at 30 min. In studies with lysed mitochondrial suspensions to which drugs are added (at concentrations of 10^{-4} M), the amount of residual 5-HT after a 15-min incubation was 44 per cent (psilocin) or 7 per cent (psilocybin) more than in the suspensions without added drug. At 10^{-5} M, the amount of 5-HT in the psilocin-treated flasks was 18 per cent more than in the control suspensions. Psilocybin at 10^{-5} M or LSD at 2×10^{-6} M had no inhibitory effect.

Figures 4 and 5 show the effects on brain 5-HT and 5-HIAA of two phenylethylamine hallucinogens, mescaline (Fig. 4) and 2,5-dimethoxy-α, 4-dimethylphenylethylamine (DOM, Fig. 5). At higher doses (5–15 mg/kg mescaline, 5 mg/kg DOM), the pattern of effects is essentially different from those of the indoleamines, in that there is an increase in brain 5-HIAA. At lower doses (1 mg or less), there is indication of an LSD-like effect (rise in 5-HT with a concomitant decrease in 5-HIAA).

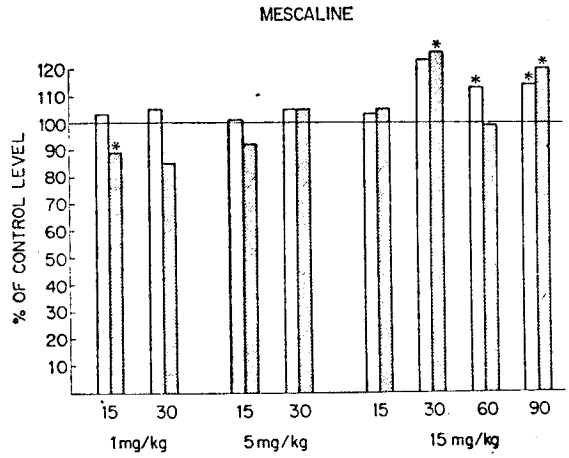

FIG. 4. The effects of different doses of mescaline on the metabolism of brain 5-HT at the times indicated (in minutes). Open bars, 5-HT; shaded bars, 5-HIAA. The changes in 5-HT and 5-HIAA are shown as per cent of the control levels at each time. Control and experimental groups had equal numbers of animals: 1 mg/kg—15 min, 6; 30 min, 10; 5 mg/kg—15 min, 6; 30 min, 6; 15 mg/kg—15 min, 12; 30 min, 4; 60 min, 10; 90 min, 6. The asterisks indicate significant changes from the control ($P < 0.05$).

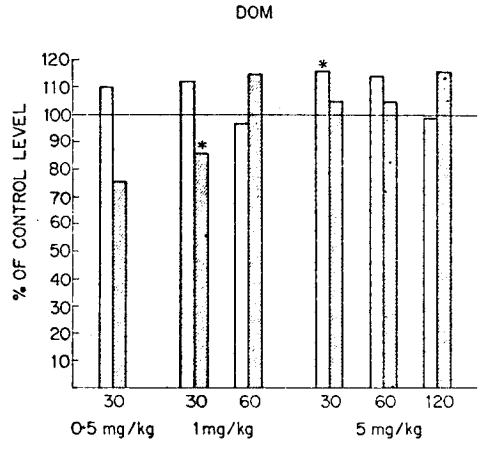

FIG. 5. The effects of different doses of 2,5-dimethoxy-α,4-dimethylphenylethylamine (DOM) on the metabolism of brain 5-HT at the times indicated (in minutes). Open bars, 5-HT; shaded bars, 5-HIAA. The changes in 5-HT and 5-HIAA are shown as per cent of the control levels at each time. Control and experimental groups had equal numbers of animals: 0·5 mg/kg—30 min, 6; 1 mg/kg—30 min, 10; 60 min, 4; 5 mg/kg—30 min, 9; 60 min, 6; 120 min, 4. The asterisks indicate significant changes from the control ($P < 0.05$).

DISCUSSION

The data clearly indicate that there is a difference in the biochemical effects of the phenylethylamine psychotomimetics (mescaline and DOM) and the indolealkylamine psychotomimetics (LSD, psilocybin and DMT), although no marked general behavioral difference has been noted. Both groups of drugs influence the metabolism of serotonin (as do the psychoactive congeners of LSD); both lead to small 5-HT increases. But the decreases of 5-HIAA were not observed with high doses of the phenylethylamine drugs. There is some indication that early in the course of their effects (15 min), or at very low dosages, levels of brain 5-HIAA decrease or are unchanged.

Among the indolealkylamines, the duration of effects on brain 5-HIAA is long. For LSD, the effects on 5-HT metabolism correlate with the time course for clearance of drug from brain, with peak effects on 5-HT and 5-HIAA corresponding with the half life of the drug in brain. Tissue levels of LSD are critical in accounting for the time course of acute behavioral effects in rat[24, 25] and in man,[26] and such time courses for the other indolealkylamine drugs have yet to be elucidated.

MAO inhibition clearly cannot be invoked to explain the effect of LSD on 5-HIAA. The effect of psilocin or psilocybin, however, may in part be explained by an effect on MAO. One difficulty with this lies in the fact that 6 hr after psilocybin, 5-HT levels are normal while 5-HIAA levels are significantly decreased. At 90 min after injection levels of norepinephrine were decreased by 24 per cent ($P < 0.001$) while 5-HT levels were increased. Thus simple inhibition of MAO is probably not the sole explanation. Satisfactory analysis of the late time course of 5-HIAA effects (60 min for DMT, at least 2 hr after LSD, and 6 hr after psilocybin) will depend not only on parameters of drug metabolism and clearance, efflux of metabolites, but, as previously suggested,[16] may also involve feedback reduction of 5-HT synthesis after earlier accumulations.

Giarman and colleagues[8, 9] early emphasized the point that levels reflected the net result of a number of component processes. These data indicate that both phenylethylamine and indolealkylamine drugs "conserve" serotonin, perhaps either by increasing synthesis or decreasing breakdown. Even these processes could be the result of a number of complex mechanisms, factors such as uptake, reuptake, binding,[7, 19] blocking and release,[28] enhanced[29, 30] or diminished[31] neural activity and drug interference with one or several amine–receptor interactions; the problem of sequences of feedback regulations may also be entailed. Apart from the specifics of biochemical sequences, their correlation with the neural activity of monoamine systems may be relevant. Aghajanian *et al.*, for example, have linked the activity of the midline raphe system with 5-HT metabolism[29, 30] and have shown that LSD can diminish firing of those neurons.[31] Mescaline diminishes firing of only a small group of these neurons with increases or no changes noted in the activity of others[32]—thus suggesting a parallel with the biochemical findings after low doses of the drug.

At present, the significance of the differences, with respect to 5-HIAA, of phenylethylamines and indolealkylamines is not clear. There is cross tolerance among them in rat[33] and it is known that increases or decreases in amine levels markedly influence the intensity of effects of LSD.[19, 34] We do not as yet know the effects of pretreatment regimens on the phenylethylamine drugs. All that is evident is that changes of serotonin metabolism, which "conserve" 5-HT,[35] are common to the two groups of psychotomimetics.

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