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STIMULATION OF [^{14}C] SEROTONIN SYNTHESIS FROM [^{14}C] TRYPTOPHAN BY MESCALINE IN RAT PINEAL ORGAN CULTURES

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Abstract. Addition of mescaline to the nutrient medium of rat pineal glands in organ culture produces a marked increase in pineal synthesis of serotonin from [^{14}C] tryptophan. By contrast, addition of psilocybin or lysergic acid diethylamide (LSD) has no effect on [^{14}C] serotonin synthesis by these cultures. Mescaline has no effect on pineal [^{14}C] serotonin synthesis when [^{14}C] 5-hydroxytryptophan is substituted for [^{14}C] tryptophan as the labelled substrate. Mescaline does not inhibit the deamination of [^{14}C] serotonin to 5-hydroxyindoleacetic acid and has no significant effect on the intracellular content of [^{14}C] tryptophan or its conversion to [^{14}C] protein. It is concluded that mescaline stimulates pineal [^{14}C] serotonin synthesis via a primary stimulatory action on the enzymatic conversion of [^{14}C] tryptophan to 5-hydroxytryptophan, rather than by effects on other steps in the indole pathway or on cellular uptake of [^{14}C] tryptophan.

The phenylethylamine and indolealkylamine hallucinogenic drugs influence the metabolism of brain serotonin in distinct but different ways. The phenylethylamine hallucinogens, mescaline and 2, 5 dimethoxy-a, 4-dimethylphenylethylamine (DOM), produce increased brain concentrations of serotonin and 5-hydroxyindoleacetic acid (5-HIAA), the deaminated metabolite of serotonin (1). In contrast, the indolealkylamine hallucinogens, psilocybin, psilocin, N, N-dimethyltryptamine (DMT) and lysergic acid diethylamide (LSD), also produce increased brain concentrations of serotonin but these drugs decrease brain 5-HIAA levels (1, 2, 3). The increased brain levels of serotonin and 5-HIAA produced by the mescaline group of

hallucinogens suggests that these drugs increase the rate of turnover of brain serotonin.

Contrariwise, the increased serotonin levels and decreased 5-HIAA levels produced by the LSD-psilocybin group of hallucinogens suggests that these drugs decrease brain serotonin turnover. Since the enzymes, tryptophan hydroxylase and aromatic l-amino acid decarboxylase (AAAD), which catalyze the biosynthesis of serotonin from the tryptophan are present in the rat pineal (4) and do not appear to differ from the tryptophan hydroxylase and AAAD in the brain (5, 6), we have used an in vitro pineal system to determine whether mescaline, LSD, or psilocybin exert a direct biochemical effect on the formation of [^{14}C] serotonin. This report will show that mescaline stimulates the synthesis of [^{14}C] serotonin from [^{14}C] tryptophan by enhancing the conversion of [^{14}C] tryptophan to [^{14}C] 5-hydroxytryptophan, and that LSD and psilocybin are without effect on pineal [^{14}C] serotonin synthesis.

Materials and Methods

Pineal glands were removed between 11 A. M. and 1 P. M. from adult female Sprague-Dawley rats previously housed under diurnal lighting conditions. Each pineal was clotted individually to the walls of a Wasserman tube, and 0. 5 ml of nutrient medium (7) was added. The nutrient medium contained [^{14}C] dl-tryptophan or [^{14}C] 5-hydroxytryptophan (0. 5mCi in a 10^{-4}M solution) and, where indicated, mescaline sulfate, psilocybin or lysergic acid diethylamide. The cultures were sealed with a rubber stopper and incubated on a roller wheel at 37°C for 48 hours. Each treatment group contained six to ten individual pineal gland cultures. Control groups contained the complete incubation mixtures but no pineal glands. At the end

of the incubation period, the nutrient media were assayed individually for [^{14}C] melatonin, [^{14}C] serotonin and [^{14}C] 5-hydroxyindoleacetic acid as previously described (8). The pineals were then analyzed for [^{14}C] protein and [^{14}C] tryptophan (9).

Results

The addition of mescaline to the nutrient medium of rat pineal organ cultures in a concentration of 10^{-6}M, caused a 300% increase in the formation of [^{14}C] serotonin from the [^{14}C] tryptophan (Table 1).

TABLE 1

Effects of Various Doses of Mescaline on Pineal Synthesis of [^{14}C] Indoles and on Pineal Content of [^{14}C] Tryptophan and [^{14}C] Protein

Mescaline Concentration (M)	[^{14}C] Serotonin (c. p. m.)	[^{14}C] Melatonin (c. p. m.)	[^{14}C] 5-HIAA (c. p. m.)	[^{14}C] Protein Content (c. p. m.)	[^{14}C] Tryptophan Content (c. p. m.)
0	557±13	305±19	515±84	263±38	265±28
10^{-6}	1596±110*	491±46#	695±86	346±41	337±69
10^{-5}	1159±208**	453±62	960±168	386±43	411±53
10^{-4}	897±109**	247±60	120±169	363±63	310±92

*$p < 0.001$, #$p < 0.02$, **$p < 0.05$. Differs from control group incubated without mescaline.

Groups of six culture tubes containing a rat pineal were incubated with [^{14}C] tryptophan (1×10^{-4}M) for two days at 37°C. Data are expressed as means ± S. E. M. of c. p. m. [^{14}C] radioactivity; 5-HIAA is 5-hydroxyindoleacetic acid.

At higher concentrations, mescaline also stimulated serotonin synthesis to a lesser extent (Table 1). In all experiments, the highest stimulation was observed with a mescaline concentration in the range 10^{-6}M to 10^{-5}M. The lesser stimulation observed with a mescaline concentration greater than 10^{-5}M (i.e., 10^{-4}M) was not due to obvious toxicity inasmuch as accumulation of [^{14}C] tryptophan and formation of [^{14}C] protein was not inhibited at this concentration (Table 1). At concentrations lower than 10^{-6}M (i.e., 10^{-7}M or 10^{-8}M), mescaline was found to have no effect on serotonin synthesis. The addition of mescaline also stimulated [^{14}C] melatonin synthesis to a slight degree, but did not produce a statistically significant change in formation of [^{14}C] 5-HIAA or in pineal content of [^{14}C] protein and [^{14}C] tryptophan (Table 1). Despite the lack of a statistically significant difference in pineal [^{14}C] protein and [^{14}C] tryptophan content between mescaline-treated and control cultures, mescaline may, nevertheless, have exerted a slight enhancing effect on these contents, inasmuch as all cultures incubated with mescaline contained more [^{14}C] protein and [^{14}C] tryptophan than controls (Table 1). However, this small effect on [^{14}C] protein and [^{14}C] tryptophan content was much too slight to account for the mescaline-induced stimulation of [^{14}C] serotonin synthesis.

For comparison with the effects of mescaline, two other hallucinogenic drugs, psilocybin and LSD, were examined for their ability to modify the synthesis of [^{14}C] serotonin and [^{14}C] melatonin in pineal organ culture (Tables 2 and 3). Neither psilocybin, in concentrations of 10^{-4}M and 10^{-5}M, nor LSD, in concentrations of 10^{-5}M and 10^{-6}M, had an effect on [^{14}C] serotonin or [^{14}C] melatonin synthesis.

TABLE 2

Lack of Effect of Psilocybin on Pineal [^{14}C] Serotonin and [^{14}C] Melatonin Synthesis

Psilocybin Concentration (M)	[^{14}C] Serotonin (c. p. m.)	[^{14}C] Melatonin (c. p. m.)
0	887±131	201±83
10^{-5}	813±95	369±47
10^{-4}	1053±113	291±34

Groups of six culture tubes containing a rat pineal were incubated with [^{14}C] tryptophan for two days at 37°C. Data are expressed as means ± S. E. M. of c. p. m. [^{14}C] radioactivity.

TABLE 3

Lack of Effect of Lysergic Acid Diethylamide (LSD) on Pineal [^{14}C] Serotonin and [^{14}C] Melatonin Synthesis

LSD Concentration	[^{14}C] Serotonin (c. p. m.)	[^{14}C] Melatonin (c. p. m.)
0	2486±198	884±67
10^{-6}M	2319±314	1034±105
10^{-5}M	2508±265	1001±72

Groups of eight rat pineal cultures were incubated with [^{14}C] tryptophan for two days at 37°C. Results are expressed as means ± S. E. M. of c. p. m. of [^{14}C] radioactivity.

To determine whether mescaline stimulates [^{14}C] serotonin synthesis by an effect at the level of tryptophan hydroxylation, comparison was made of the effect of mescaline when [^{14}C] 5-hydroxytryptophan was substituted for [^{14}C] tryptophan as the substrate for [^{14}C] serotonin synthesis in the nutrient medium (Table 4). In contrast to a marked mescaline-induced

TABLE 4

Lack of Effect of Mescaline on [^{14}C] Serotonin and [^{14}C] Melatonin Synthesis when Pineals Incubated with [^{14}C] 5-Hydroxytryptophan instead of [^{14}C] Tryptophan

Labelled Substrate		Mescaline	[^{14}C]	[^{14}C]
[^{14}C] Tryptophan	[^{14}C] 5-Hydroxytryptophan	Concentration (M)	Serotonin (c. p. m.)	Melatonin (c. p. m.)
0	+	0	4151± 182	1016± 66
0	+	10^{-5}	3755± 162	922± 52
+	0	0	1610± 230	827± 93
+	0	10^{-5}	4133± 510*	1226± 86**

* $P < 0.002$, ** $P < 0.02$, differs from cultures incubated with [^{14}C] tryptophan but without mescaline.

Groups of ten pineal cultures were incubated with [^{14}C] tryptophan ($1x10^{-4}$M), or [^{14}C] 5-hydroxytryptophan ($1x10^{-4}$M), in the presence or absence of mescaline for two days at 37°C. Data are expressed as means ± S. E. M. of c. p. m. [^{14}C] radioactivity.

stimulation of [^{14}C] serotonin and [^{14}C] melatonin synthesis observed in parallel cultures incubated with [^{14}C] tryptophan, no effect of mescaline was observed on [^{14}C] serotonin or [^{14}C] melatonin synthesis in pineal cultures incubated with [^{14}C] 5-hydroxytryptophan.

Discussion

The present observations demonstrate that mescaline increases the formation of [^{14}C] serotonin from [^{14}C] tryptophan by rat pineal glands in organ culture. In contrast, LSD and psilocybin have no effect on [^{14}C] serotonin synthesis in rat pineal cultures.

The mechanism whereby mescaline increases pineal synthesis of [^{14}C] serotonin from its amino acid precursor appears to involve an action(s) primarily at the site of tryptophan hydroxylation (Fig. 1).

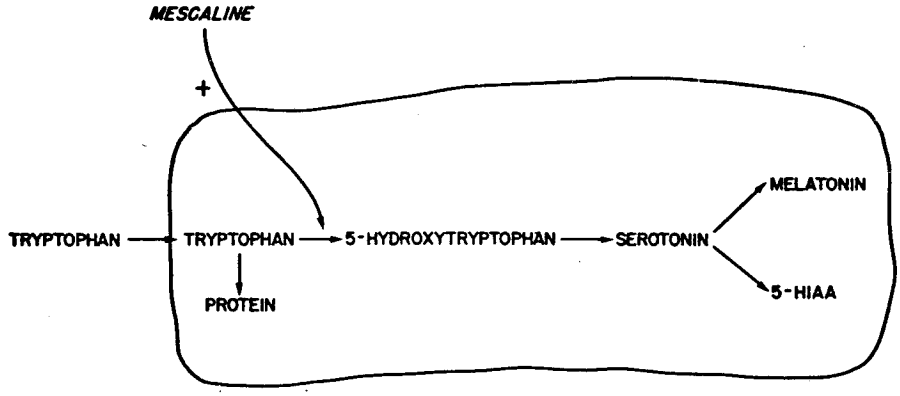

FIG. 1

Uptake and metabolism of tryptophan in pineal organ culture and site of stimulatory action (+) of mescaline.

Mescaline does not stimulate pineal [^{14}C] serotonin (or [^{14}C] melatonin) synthesis when the hydroxylation step is bypassed by substitution of [^{14}C] 5-hydroxytryptophan for [^{14}C] tryptophan as the labelled substrate. Mescaline does not stimulate [^{14}C] serotonin concentration in the pineal

cultures via inhibition of serotonin destruction by monoamine oxidase since mescaline has no effect on [^{14}C] 5-HIAA formation. Mescaline does not stimulate pineal [^{14}C] serotonin formation primarily by a "pool" effect, via stimulation of the cellular uptake of labelled tryptophan, since mescaline has at most a very slight effect on the intracellular content of [^{14}C] tryptophan and on the accumulation of intracellular [^{14}C] protein. The precise mechanism(s) by which mescaline stimulates the enzymatic conversion of tryptophan to 5-hydroxytryptophan has not yet been identified. However, the stimulation by mescaline probably does not involve the intermediary action of cyclic AMP inasmuch as dibutyryl cycle AMP (DAMP), unlike mescaline, produces a proportionally much greater stimulation of [^{14}C] melatonin than of [^{14}C] serotonin synthesis (10), and DAMP achieves at least part of its stimulatory effect on [^{14}C] melatonin synthesis at a different locus than tryptophan hydroxylation, i. e., by stimulation of the N-acetylation of serotonin (11).

The differences observed in the present study between the *in vitro* effects of mescaline and LSD or psilocybin on [^{14}C] serotonin synthesis are consistent with previously observed differences between the effects of mescaline and LSD or psilocybin on whole rat brain concentrations of serotonin and 5-HIAA *in vivo*. Mescaline produces increased brain concentrations of both serotonin and 5-HIAA *in vivo* (1), and produces increased synthesis of [^{14}C] serotonin without associated change in [^{14}C] 5-HIAA formation in pineal glands *in vitro*. Considered together, the *in vitro* and *in vivo* observations on the effects of mescaline are compatible with the hypothesis that mescaline elevates brain serotonin and 5-HIAA levels by stimulating the synthesis of serotonin from tryptophan in serotonin-containing neurons. Psilocybin and LSD produce increased

concentrations of brain serotonin and decreased concentrations of brain 5-HIAA *in vivo* (1, 2, 3, 12), but produce no effect on [^{14}C] serotonin synthesis in pineal glands *in vitro*. Considered together, these *in vitro* and *in vivo* observations on the effects of LSD and psilocybin support the suggestion of Freedman and co-workers (1) that these hallucinogens do not elevate brain serotonin levels by stimulating serotonin synthesis but rather by "conserving" brain serotonin in serotonin-containing neurons. Consistent with this concept of "conserving" effect of LSD and psilocybin are the observations of Aghajanian and co-workers that LSD can diminish the firing of midline raphe serotonin-containing neurons (2) while, in contrast, mescaline diminishes firing of only a small group of these neurons and increases or leaves unchanged the activity of others (13).

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References

1. Freedman, D. X., R. Gottlieb and R. A. Lovell, Biochem. Pharmac., 19, 1181 (1970).

2. Freedman, D. X. and G. K. Aghajanian, Lloydia 29, 309 (1966).

3. Diaz, P., S. H. Ngai and E. Costa, Pharmacologist 9, 372 (1967).

4. Wurtman, R. J., J. Axelrod and D. E. Kelly, The Pineal, Academic Press, N. Y. (1968).

5. Lovenberg, W., E. Jequier and A. Sjoerdsma, Science 155, 217 (1967).

6. Snyder, S. H., J. Axelrod, R. J. Wurtman and J. E. Fischer,

J. Pharmac. Exp. Ther. 147, 371 (1965).

7. Shein, H. M., R. J. Wurtman and J. Axelrod, Nature 213, 730, (1967).

8. Wurtman, R. J., F. Larin, J. Axelrod, H. M. Shein and K. Rosasco, Nature, 217, 953, (1968).

9. Wurtman, R. J., H. M. Shein, J. Axelrod and F. Larin, Proc. Nat. Acad. Sci. U. S. 62, 749 (1969).

10. Shein, H. M. and R. J. Wurtman, Science, 166, 519 (1969).

11. Klein, D. C., G. R. Berg and J. Weller, Science, 168, 979, (1970).

12. Aghajanian, G. K., W. E. Foote and M. H. Sheard, Science, 161, 706, (1968).

13. Aghajanian, G. H., M. H. Sheard and W. E. Foote, in Psychotomimetics (Ed. D. H. Efron), Raven Press, N. Y., in press.