Biochemical Pharmacology, Vol 24, pp. 929-932. Pergamon Press, 1975. Printed in Great Britain.

PRELIMINARY COMMUNICATION

ENZYMATIC FORMALDEHYDE PRODUCTION FROM 5-METHYLTETRAHYDROFOLIC ACID: PRIOR STEP TO ALKALOID FORMATION*

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(Received 3 January 1975; accepted 31 January 1975)

5-Methyltetrahydrofolic acid was recently reported to mediate the N-methylation of dopamine to epinine in rat brain.[1] Various properties of this enzymatic reaction have since been described,[2-7] enabling us to attribute a more general function to this reaction than a function involving only the adrenergic system.[8]

Two main features prompted us to undertake this investigation. Firstly, in contrast to other studies[4, 5] we did not identify the corresponding N- or O-methylated compounds when tryptamine, N-methyltryptamine or 5-hydroxytryptamine were used as substrate.[6] Similar results were recently obtained by another group.[9] Secondly, blank values differed markedly, depending on the procedure of isolating reaction products. This suggested, that something other than a simple methyl transfer was involved.

We tested two enzyme preparations in this study: one from rat kidney and another from pig brain. The radioactivity measured in extracts from reaction mixtures previously incubated with 5-Me^{C14}-H_4-folate and two enzyme fractions isolated from rat kidney, differed markedly, depending on how the blank was run (Table I). Although all the blank values were lower than those of the assay, the addition of substrate (4-methoxy, 3-hydroxyphenylethylamine) after the incubation mixture was brought to pH 10, caused a tremendous increase in the radioactivity. In contrast to this, the blank containing boiled enzyme gave lower values (even lower than those for the blank without substrate). In fact that after evaporation, the amount of radioactivity decreased much more in the blank without substrate than in that where the substrate was added after

* Part of this work was presented at the IXth C.I.N.P. Congress, Paris, July 1974

TABLE I. Radioactivity extracted from reaction mixtures incubated with 5-Me^{C14}-H_4-folate and purified enzyme fractions from rat kidney

	Total extracted radioactivity (CPM x 10^3)	
	Peak A	Peak B
Before evaporation		
Assay	19.1	33.4
Blank		
Boiled enzyme	1.4	2.5
Without substrate	2.1	3.8
Substrate added after fixation	8.4	18.8
After evaporation		
Blank		
Without substrate	0.3	0.2
Substrate after fixation	8.4	7.6

Ammonium sulphate enzyme fraction (30 % to 60 %) from rat kidney was further purified on DEAE A50 Sephadex columns as described elsewhere.[9] An aliquot of two peaks (A and B) of enzyme activity was incubated with 0.1 µCi 5-Me^{C14}-H_4-folate (spec.act. 61 mCi/mmol Radiochemical Centre Amersham) and the reaction products were extracted as previously described.[6]

fixation, suggested that, during the incubation period, a volatile product was formed. Moreover, this product must have a very high affinity for the amines; a reaction seemed to occur with the substrate added after fixation. In accordance with this hypothesis, formaldehyde production was recently demonstrated using the dimedon precipitation method,[10] in reaction mixtures incubated with 5-Me-H_4-folate and rat kidney enzyme but in the absence of biogenic amines.[11] These data were now confirmed in the presence of substrate like 4-methoxy, 3-hydroxyphenylethylamine. The free formaldehyde enzymatically formed was found to decrease tremendously if the amine was added to the incubation mixtures suggesting a possible condensation reaction between aldehyde and amine. This view is supported by experiments where formaldehyde^{C14} was only incubated at 0° C with a biogenic amine but without enzyme. Here again, the presence of the amine considerably lowered the amount of formaldehyde precipitated with dimedon.

These experiments clearly demonstrated not only that formaldehyde was enzymatically formed from 5-Me-H_4-folate as reported in detail elsewhere,[11] but also that an additional reaction occurred between the aldehyde and amine. A first approach in elucidating the nature of this condensation product is presented in figure 1. By incubating epinine with a pig brain enzyme, an alkaloid, 2-methyl 6, 7-hydroxy 1, 2, 3, 4-tetrahydroisoquinoline was identified by thin-layer chromatography (fig. 1). Similar results were obtained with other amines

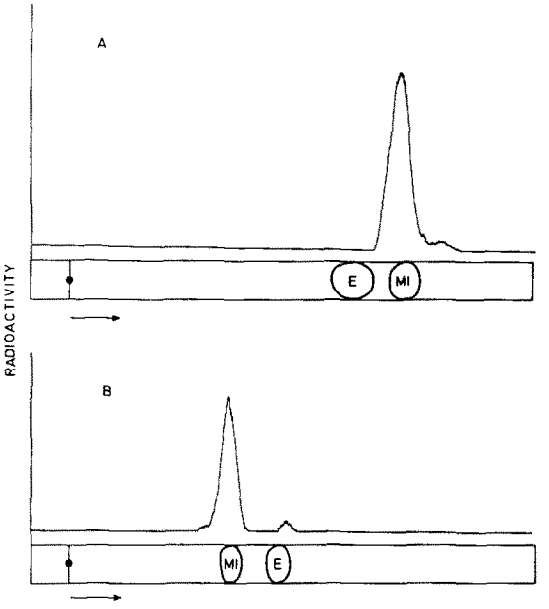

Fig. 1 - Radiochromatograms of a reaction mixture incubated with 5-Me^{C14}-H_4-folate, epinine and an ammonium sulphate enzyme fraction (30 to 65 per cent) from pig brain. Reaction products were isolated on Al_2O_3 columns.[6] An aliquot was spotted on (A) cellulose F and (B) silica gel 60 F plated. Solvent systems: (A) phenol-water-HCl 80/20/1 (B) isobutyl alcohol-formic acid-water 150/30/20. Radioactivity was measured with a Berthold scanner and standards (E: epinine and Mi: 2-methyl 6, 7-hydroxy 1, 2, 3, 4-tetrahydroisoquinoline) were detected in U.V.

like tryptamine, N-methyltryptamine and 4-methoxy, 3-hydroxyphenylethylamine using five different solvent systems.[12] Moreover the identity of the respective reaction products was further proved by gas chromatography and mass fragmentography as it will be reported in detail elsewhere.[12] This explains the earlier experiments[6, 9] in which N-methylation of indoleamines could not be obtained but does not confirm the suggestion of a possible methylation of the indole nitrogen.[9]

Therefore, the foregoing results support the view that alkaloids of the tetrahydroisoquinoline or the β-carboline group could be formed <u>in vivo</u> by the Pictet-Spengler condensation of corresponding amines with formaldehyde as recently postulated.[13] A similar condensation reaction of dopamine with acetaldehyde (yielding salsolinol) has been demonstrated <u>in vivo</u> in the urine of parkinsonian patients being treated with L-Dopa[13] although it has not yet been detected in brain. Furthermore, our results provide evidence for the occurrence of a formaldehyde-forming enzyme which is present in numerous tissues and the properties of which seem more or less similar to those of an enzyme already described.[14] As shown in fig. 2, the formation of certain alkaloids

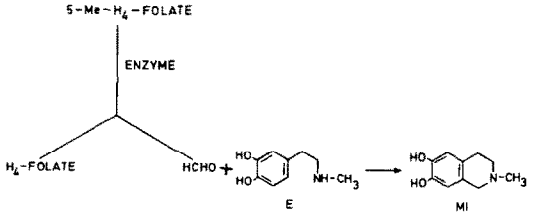

Fig. 2 - Model illustrating the alkaloid formation from biogenic amines through a one-carbon transfer from 5-Me-H_4-folate with formaldehyde as intermediate compound.

would involve a two-step phenomenon, the first one occurring enzymatically and the second one by a condensation reaction. Among the alkaloids derived from indoleamines, harmaline and harmine are known to be potent hallucinogens while certain tetrahydroisoquinolines derived from dopamine were reported as inhibitors of neuroamine metabolism[15] and of catecholamine uptake[16] although in both cases only at high concentrations. As these compounds are most probably not degraded by MAO, their action might be much more prolonged than that of their corresponding amines.

Whether these alkaloids derived from catecholamines, tryptamine, serotonin and other phenylethylamines are really synthesized in vivo, remains to be elucidated. Nevertheless, we can hypothesize that these compounds might be produced in abnormal amounts in pathological conditions, and perhaps even in schizophrenic disorders.

This work was partially supported by a grant from IRSIA. The skillful technical assistance of W. Gommeren, S. Aerts and M. Verwimp is greatly appreciated. We also thank H. Verhoeven for synthesis of compounds.

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