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PRELIMINARY COMMUNICATIONS

MASS SPECTROMETRIC IDENTIFICATION OF N-MONOMETHYLTRYPTAMINE FOLLOWING INCUBATION OF TRYPTAMINE WITH BRAIN PROTEIN AND S-ADENOSYLMETHIONINE OR 5-METHYTETRAHYDROFOLIC ACID

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In another report (1) we have shown that a radiochemical assay (9) of indoleamine N-methyltransferase activity in brain tissue using S-adenosylmethionine (SAM) as putative methyl donor and tryptamine as acceptor is unsatisfactory since the majority of the radioactive product used for quantification of the activity is not an N-methylated derivative of tryptamine. A similar assay using 5-methyltetrahydrofolate (5-MTHF) as putative donor of CH_3 groups has been shown to yield β-carboline derivatives rather than N-methylated indoleamines as the main products (2-7). These results call into question the widely quoted reports of N-methylating activity towards indoleamines in the brain using radiochemical assays (for example see 8 and 9). As we and others have shown, however, the radiochemical assay can be used to demonstrate the existence in preparations of rabbit lung of an enzyme utilizing SAM for the N-methylation of tryptamine. The question as to whether indoleamine N-methyltransferase activity occurs in brain is of course of considerable interest due to the hallucinogenic properties of N,N-dimethyltryptamine (DMT) in man (10).

In this communication we present an assay for tryptamine N-methylation using a gas chromatography/mass spectrometry technique (GC-MS) which shows that while some SAM-dependent N-methylation of tryptamine does apparently occur using preparations of rat brain, the concentration is very much lower than previously supposed. We have also confirmed by the same technique previous reports (11, 12) that the major product of the SAM-dependent reaction with tryptamine and protein from rabbit lung is N-methyltryptamine (NMT). Further, our data show that under the same incubation conditions, but with 5-MTHF instead of SAM as donor, the products of the reaction include N-monomethyltryptamine (NMT).

Methods

A Finnigan 3200F GC-MS instrument incorporating a Finnigan 6100 data system was used in conjunction with a 5 ft column of 3% OV-17 (80-100 mesh). Injection temperature was 250°C, GC-oven temperature was 170°C or 180°C (isothermal), transfer line 280°C, separator oven 260°C (carrier gas, methane, with a flow rate of 15 ml/min). Mass spectrometry used

chemical ionisation (reactant gas, methane) source pressure 1 Torr, source temperature 120°C, ionisation energy 100 eV, filament current 0.7 mA, preamplifier sensitivity 10^{-9} and electron multiplier 2 kV. We are grateful to the Finnigan Corporation, Hemel Hempstead, Herts, U.K. for allowing us access to their equipment and to Dr. S. Evans of the Finnigan Corporation for assistance and guidance in its use.

The presence of NMT and DMT was sought in reaction products using both 5-MTHF and SAM as putative donors, and dialysed preparations of lung protein from a rabbit and a dialysed brain preparation from rat (1). Incubation conditions were 2 h at 37°C with 5 mM-tryptamine and 30 μM-SAM or 5-MTHF. The medium was buffered with 0.01 M-sodium phosphate (pH 7.9 when using SAM and pH 7.0 using 5-MTHF). The reaction was stopped by addition of 0.5 M-borate buffer (pH 7.0). A small amount of 5-methyl-N,N-dimethyltryptamine was added as carrier and standard. Following extraction of the products into toluene (1) the solvent was evaporated to a fraction of its original volume. Ethanol was then added and the evaporation process repeated twice. Finally the extract, now almost completely in ethanol, was taken down to a volume of 200-300 μl. Immediately before the GC-MS run, the ethanol was evaporated to dryness under a stream of N_2. Heptane (50 μl) followed by 25 μl of trifluoroacetic anhydride was added. The vessel was then stoppered and heated for 30 min at 60°C. The extract was dried under N_2 and the residue dissolved in 20 μl of ethyl acetate. This solution (in 1 μl samples) was used for injection into the column inlet.

Results and Discussion.

By monitoring the mass of the pseudo-molecular ion it was shown that incubation of both brain and lung preparations with SAM and tryptamine gave rise to a substance with the same mass ion (m^+/e) and the same retention time as the authentic NMT-derivative (Fig.1). The amount of NMT estimated by this means to be present in the extractable product using the lung preparation and SAM as donor indicated a rate of transferase activity of 850 pmol of NMT/mg of protein/h compared to a rate of 1000 pmol/mg/h using the radiochemical method. In the case of the brain preparation the rate calculated from GC-MS analysis was very much lower, amounting to less than 2 pmol of NMT/mg of protein/h and less than 20% of the rate assayed radiometrically. In both these cases the presence of traces of the DMT-derivative could not be discounted, as small peaks at the correct mass and retention time would have been masked by the presence of other material of the same mass but similar retention time. An NMT-derivative peak at the correct mass in the case of the lung preparation was also observed with 5-MTHF as donor; quantitative analysis suggested a rate of NMT formation of 640 pmol/mg of protein/h.

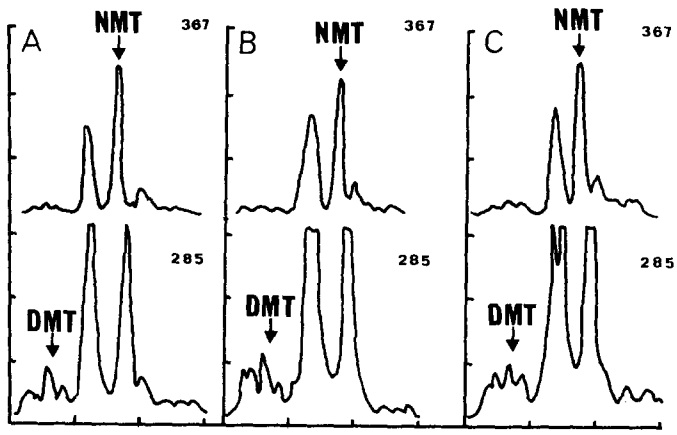

Fig. 1. Pseudo-molecular ion monitoring for NMT and DMT derivatives from incubations of tryptamine with A, rabbit lung with SAM as donor; B, rat brain with SAM and C, rabbit lung with 5-MTHF. The plots are of signal strength against retention time in arbitary units; m^+/e for the NMT derivative is 367 and for the DMT derivative is 285. The effluent was also simultaneously monitored (plot not shown) at m^+/e 299 for the internal standard of derivatised 5-methyl-N,N-dimethyltryptamine. A standard run of a mixture of the NMT, DMT and 5-methyl-N,N-dimethyltryptamine derivatives established the retention times to be expected. In practice the position (indicated by arrows) of the derivatives in the experimental effluents was determined by reference to the internal standard as the absolute retention times varied slightly from run to run.

To obtain further evidence for the formation of NMT using 5-MTHF as the source of methyl groups, multiple ion detection was used. From the total mass spectrum of a sample of the authentic NMT-derivative the three major ion fragments were identified by their mass. During a subsequent run of the authentic derivative the mass spectrometer was focussed simultaneously on the pseudo-molecular ion (m^+/e 367) and the three fragments (m^+/e, 407, 395 and 240), and for each fragment the ratio of its peak height to that of the parent ion was measured and recorded. A sample of the product formed by the incubation of 5-MTHF and tryptamine with lung protein under the usual conditions was then run on the column and the effluent monitored for the pseudo-molecular ion of the NMT-derivative and its three fragments. It was found that for two of the fragments (m^+/e, 407 and 397) the peak height ratios were identical to those observed in the standard run; in the case of the fragment of m^+/e 240 the ratio could not be determined owing to overloading.

The very low amount of N-methylation of tryptamine with SAM as donor in brain extracts is consistent with our other report (1) and with the work of Taylor and Hanna (6). However until the pathway has been shown unequivocably to operate in the C.N.S. *in vivo* the physiological significance of the *in vitro* observations will remain dubious. Moreover our experiments do not exclude the possibility of some non-enzymic formation of NMT occurring in the reaction mixture, possibly from traces of formaldehyde produced during incubation; this source of NMT is considered unlikely, however, since the non-enzymic N-methylation of

primary amines by formaldehyde only occurs under acid conditions (13).

The demonstration of a relatively high rate of tryptamine N-methylation using 5-MTHF and lung protein was unexpected in view of other work employing radiochemical assays (1, 5) discounting the existence of this reaction. In view of the fact that lung preparation used in the present work had been extensively dialysed we tend to discount the possibility that labelled CH_3 groups from 5-MTHF were first exchanged by some unknown mechanism with endogenous SAM molecules, before transfer to tryptamine. Further work is required to determine whether the system utilizing 5-MTHF is a separate entity from that utilizing SAM for transferring methyl groups.

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