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# ABSENCE OF 3-METHOXY-4-HYDROXYPHENYLETHANOL IN BRAIN*

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**Abstract**—The occurrence of measurable quantities of 3-methoxy-4-hydroxyphenylethanol (MHPE), a minor metabolite of dopamine, in brain and CSF is controversial. Utilizing gas–liquid chromatography and electron capture detection in a study of trifluoroacetyl and pentafluoropropionyl derivatives of this compound, we were unable to confirm the presence of MHPE in either whole brain or striatal tissue.

3-METHOXY-4-HYDROXYPHENYLETHANOL (MHPE) is a minor metabolic product of dopamine. Rutledge and Jonason[1] showed that rabbit brain cortex slices incubated with [$^{14}$C]dopamine produced primarily homovanillic acid and dihydroxyphenylacetic acid. The two alcoholic products, MHPE and dihydroxyphenylethanol, were formed in very small amounts. In a similar study of rat brain slices incubated with [$^{14}$C]dopamine, Breese *et al.*[2] found that 90 per cent of the metabolized dopamine was recovered in the acid fraction, whereas only 0·8 per cent was recovered in the neutral fraction. In humans, alcohol metabolites account for only 1 per cent of the total metabolites isolated following the intravenous administration of [$^{14}$C]dopamine.[3]

There is controversy concerning the detectability of endogenous levels of MHPE in brain and CSF. Ashcroft *et al.*[4] were unable to detect MHPE in the CSF drawn from the lateral ventricle of the dog. Fluorimetric procedures, however, may not possess the requisite sensitivity for the detection of the anticipated low levels of MHPE. This compound, possessing hydroxyl groups, can be analyzed by gas–liquid chromatography coupled with electron capture detection after derivatization with a halogenated anhydride.[5] Utilizing this technique, Wilk[6] was unable to detect MHPE in human CSF and estimated its concentration to be less than 1·5 ng/ml. However, Karoum *et al.*[7] reported MHPE levels of 5–10 ng/ml in CSF and 39–53 ng/g of rat brain. MHPE has also been reported to be present in human CSF and rat brain by Waterbury *et al.*[8,9] and, more recently, in rat brain by Braestrup[10] (108 ng/g, uncorrected for recovery). While studying levels of 3-methoxy-4-hydroxyphenylglycol in CSF and brain,[11] we were unable to detect MHPE in any samples of CSF or brain; our method is sensitive enough to have revealed MHPE even in the lower levels[7] reported. The presence of a peak with the retention time of an MHPE derivative on a single column is obviously not proof of identity. In an effort to clarify these discrepancies, we set out to reproduce the conditions outlined by Braestrup.[10]

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## MATERIALS AND METHODS

Three % OV-17 coated on gas chromosorb Q 80/100 mesh, 2·5% OV-17 coated on chromosorb G 80/100 mesh, as well as other coated gas chromatography packing materials were obtained from Applied Science Laboratories. Pentafluoropropionic anhydride was obtained from Pierce Chemical Company, trifluoroacetic anhydride from Eastman Organic Company, pesticide grade ethyl acetate from Analabs, and glusulase from Endo Corp. Striatal tissue was dissected from bovine, sheep and pig brains obtained on ice from a local slaughterhouse. Whole brains of male Sprague–Dawley rats were obtained after decapitation or cervical dislocation.

*Analysis of MHPE by trifluoroacetylation.* Tissues were homogenized in 10 vol. of cold 0·4 N perchloric acid and centrifuged in a Sorvall refrigerated centrifuge. The supernatant material was adjusted to pH 5·5 with 1 M $K_2CO_3$ and centrifuged again to remove potassium perchlorate. The supernatant material was incubated overnight at 37° with 0·05 vol. of glusulase. In some cases, glusulase was dialyzed against 5 mM phosphate buffer, pH 7·2 for 12 hr before use. A 4-ml sample of the hydrolyzed brain supernatant material was extracted twice with 8 ml ethyl acetate, and the pooled ethyl acetate extracts were evaporated to dryness in a 100-ml round-bottomed flask with a flash evaporator. To the flask were added 1 ml pesticide grade ethyl acetate, 0·25 ml trifluoroacetic anhydride and 0·01 ml of 0·1 M tripropylamine in benzene (catalyst). The flask was stoppered with a ground-glass stopper, the outside sealed with parafilm to protect against moisture, and the flask immersed in a 50° water bath for 30 min. After reaction, the flask was allowed to cool to room temperature and the contents evaporated under a stream of $N_2$. To the dried flask was added 1 ml of pesticide grade ethyl acetate, the contents were dissolved and transferred to a small aluminium foil-lined screw-capped vial for storage prior to chromatography. Parallel samples containing added MHPE together with reagent blanks containing $H_2O$ in place of the brain supernatant were processed with the unknown. Chromatography of 5-$\mu$l samples was carried out on a Packard 7400 series gas chromatograph fitted with a 150-mCi tritium foil electron capture detector.

*Analysis of MHPE by treatment with pentafluoropropionic anhydride.* The procedure of Braestrup[10] was exactly reproduced. This method differs from that described above in several respects: bisulfite and EDTA are added to the perchloric acid; rat brain is homogenized in a much smaller volume; glusulase is incubated at pH 6; pentafluoropropionic anhydride is reacted at room temperature without a catalyst.

## RESULTS

When brain samples were treated with trifluoroacetic anhydride as described and chromatographed on 3% OV-17 coated on gas chromosorb Q at 120°, there was no evidence of any MHPE in the samples. When MHPE (50–200 ng) was added to brain samples and carried through the procedure, a peak at the retention time of MHPE was observed. Under these conditions, it was estimated that if MHPE is present in brain it exists in amounts less than 25 ng/g. Striatal tissue of several species was treated in a similar manner and in none could MHPE be detected; if present, its concentration is less than 25 ng/g striatum (Fig. 1; Table 1).

When brain samples were treated with pentafluoropropionic anhydride as described by Braestrup[10] and chromatographed on 2·5% OV-17 coated on chromosorb

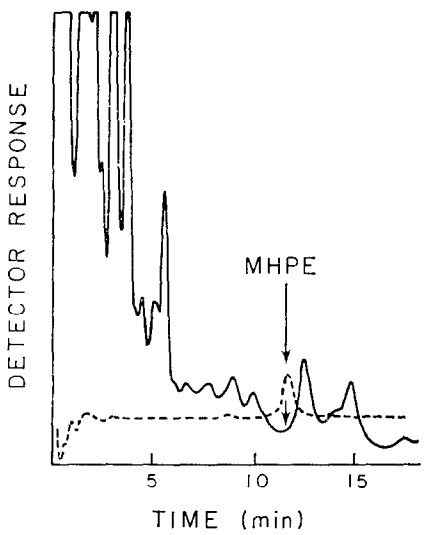


FIG. 1. Beef striatum (0·38 g) assayed for MHPE by trifluoroacetylation. The retention time of MHPE trifluoroacetate is shown by the curve traced with a dashed line. 3% OV-17 on gas chromosorb Q 60/80 mesh, 120° (Final volume, 1 ml, 5 $\mu$l injected onto column).

G at 150° and the flow rate adjusted so as to yield a retention time of the MHPE derivative of exactly 10 min, a peak in the brain samples was observed at a retention time (RT) slightly longer than that of MHPE (RT = 10·6 min, Fig. 2A). Addition of 250 ng MHPE to parallel samples led to an almost total overlap of the two peaks, thus resulting in a potentially misleading interpretation (Fig. 2B). The asymmetry of the overlapping peak was revealed when smaller amounts of MHPE (50 ng) were added to a parallel brain sample (Fig. 2C).

Chromatography of these same samples on the gas chrom Q column at 120° revealed an interfering peak at a retention time slightly shorter than that of the MHPE

TABLE 1. TISSUES ASSAYED FOR MHPE

| Sample | Derivative | Results (ng/g) |
|---|---|---|
| Whole guinea-pig brain | PF* | < 10 |
| Whole rat brain | PF | < 10 |
| Whole rat brain | TF† | < 25 |
| Sheep striatum | TF | < 25 |
| Pig striatum | TF | < 25 |
| Beef striatum | TF | < 25 |
| Beef striatum | PF | < 10 |

* PF = pentafluoropropionyl derivative.
† TF = trifluoroacetyl derivative.

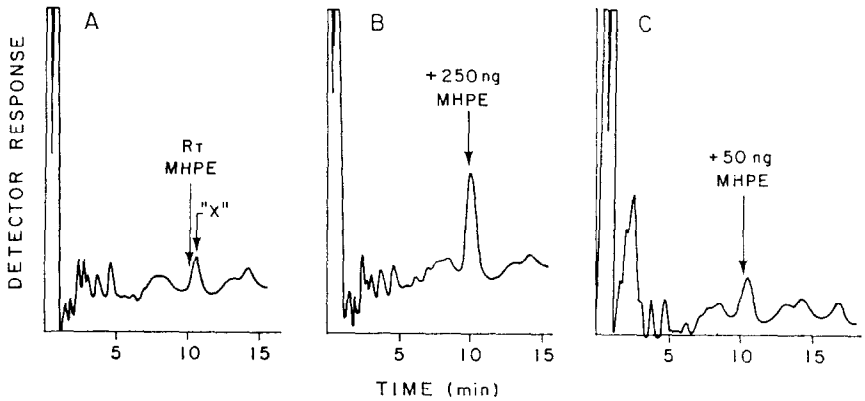


FIG. 2. (A) Whole rat brain (1·07 g) treated as described by Braestrup.[10] 2·5% OV-17 coated on chromosorb G 80/100 mesh, 150°. Final volume, 2 ml; inject 2 μl. (B) Whole rat brain (1·32 g) with 250 ng MHPE added at start of procedure. Chromatographic conditions as above, (C) Whole rat brain (1·47 g) with 50 ng MHPE added at start of procedure. Chromatographic conditions as above.

derivative; lowering the column temperature to 110° almost totally resolved the two peaks. In the native brain sample there was no evidence of any MHPE, whereas when 50 ng MHPE had been added at the beginning of the procedure a peak for the MHPE-pentafluoropropionate derivative was clearly shown (Fig. 3). Under these conditions, we can conservatively estimate that if MHPE exists in brain its concentration is less than 10 ng/g.

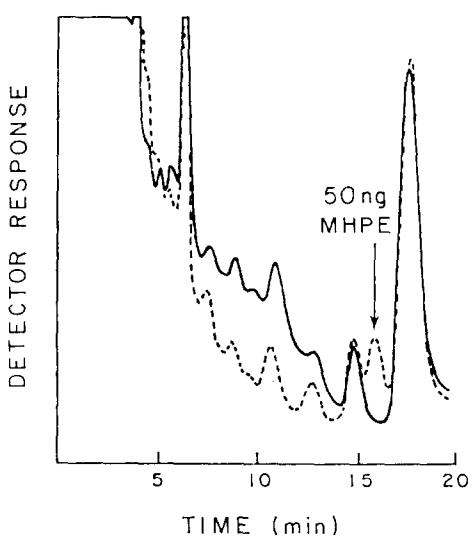


FIG. 3. Whole rat brain (1·32 g) treated as described by Braestrup[10] (solid line). Whole rat brain (1·47 g) plus 50 ng MHPE added at start of procedure (dashed line). 3% OV-17 on gas chromosorb Q, 60/80 mesh, 110°. Final volume, 2 ml; 8 μl injected onto column.

## DISCUSSION

The amount of MHPE formed from dopamine as measured by radioisotope studies both *in vivo* and *in vitro* represents only a very small fraction of the total metabolites.[1–3] Tissue levels of this compound would therefore be expected to be very low. By employing trifluoroacetylation to yield a derivative of MHPE to which the electron capture detector responds quite sensitively, we confirmed our earlier impression that MHPE if present in brain or CSF is present in levels below the limit of sensitivity of our method. We then set out to reproduce the conditions of Braestrup,[10] who reported MHPE levels of 108 ng/g of brain. Again we were unable to detect MHPE. A peak was observed, however, with a retention time almost identical to that of MHPE.

The addition of relatively large amounts of MHPE (250 ng) to parallel brain samples produced a chromatogram in which the combined peak of unknown and standard presented a symmetrical appearance (Fig. 2B). Braestrup[10] added a 500 ng internal standard in his studies. Only by lowering the amount of internal standard to 50 ng could the asymmetry be apparent (Fig. 2C). Moreover, by substitution of a gas chromosorb Q column and by lowering the temperature to 110° we were able to effect an almost complete resolution of the unknown and MHPE standard. MHPE was absent in brain samples, whereas a parallel sample containing 50 ng of added MHPE yielded a well-defined peak for the pentafluoropropionyl derivative (Fig. 3).

The determination of MHPE in rat brain stem and cortex using a pentafluoropropionyl derivative and electron capture detection was described in an abstract by Wendel *et al.*[9] Free MHPE was apparently measured, but levels were not reported. MHPE in brain and CSF has also been reported by Waterbury and Pearce[8] and by Karoum *et al.*[7] In both cases, the published chromatograms show no clear resolution of the "MHPE peak" from other components. Waterbury and Pearce[8] reproduced a mass spectrum of the trimethylsilyl derivative of MHPE isolated from CSF. We are unable to account for this observation. Using an electron capture detector, we observed[6] that the total MHPE in CSF exists at levels less than 1·5 ng/ml. Waterbury and Pearce[8] used the hydrogen flame detector, which is 100- to 1000- times less sensitive than the electron capture detector and detected MHPE in samples of 2 $cm^3$ CSF that had not been treated with glusulase.

In the gas chromatographic studies of Braestrup,[10] identification of MHPE was based upon the identical retention time of the MHPE–pentafluoropropionyl standard and the unknown peak utilizing a single column. Similar results were obtained for the heptafluorobutyryl derivative of MHPE on the same column. These results were further supported by the identical properties of MHPE and the unknown, utilizing thin-layer chromatography and paper chromatography.

The use of a single column for characterization of an unknown compound is, of course, not satisfactory. Substitution of a heptafluorobutyryl for a pentafluoropropionyl derivative may not be helpful, since these derivatives have similar chromatographic properties.[5] Comparison of the retention time of unknown and standard should be carried out using a series of columns coated with liquid phases of differing degrees of polarity. Moreover, the chromatographic conditions utilizing a single column should be varied. The temperature dependence of retention time for two substances may differ. Thus two substances which give coincident peaks at one temperature may be at least partly resolved at another temperature. The amount of

added internal standard should be small enough so that any asymmetry resulting from a combination of unknown and internal standard can be observed.

The absence of detectable MHPE in striatal tissue from three species (Table 1) lends further credence to its status as a minor metabolite of dopamine. In view of these observations, we feel that the occurrence of MHPE in the central nervous system in measurable quantities is yet to be demonstrated.

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## NOTE ADDED IN PROOF

In a recent report B. L. Goodwin *et al.* (*Clin. Chim. Acta* **40,** 269 (1972), noted that an interfering peak in their enzyme preparation has a retention time identical with that of MHPE. They concluded that the relatively low values which they previously reported—39–53 ng/g rat brain (Karoum *et al.*[7])—are likely to have been too high.