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SHORT COMMUNICATIONS

Gas chromatographic evidence for the presence of 3-methoxy-4-hydroxyphenylethanol in rat brain

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THE ACTION of monoamine oxidase on phenylethylamines results in the formation of the corresponding aldehydes.[1] In the tissues these can either be reduced by an aldehyde reductase or further oxidized to acids by aldehyde dehydrogenase. 3,4-dihydroxyphenylglycolaldehyde, which is formed after deamination of norepinephrine,[2] is probably mainly reduced and O-methylated to 3-methoxy-4-hydroxyphenylglycol (MOPEG) in brain tissues,[3-5] while it is generally believed that the main cerebral metabolite of dopamine is homovanillic acid, the oxidized product.

However, when radioactive L-Dopa, a precursor of dopamine, or dopamine itself is administrated to rats *in vivo*, appreciable amounts of labelled 3-methoxy-4-hydroxyphenylethanol (MOPET) is found in the brain, especially after inhibition of aldehyde dehydrogenase with calcium carbamide.[6,7] Preliminary experiments in this laboratory using labelled tyrosine as precursor of dopamine and norepinephrine have shown that radioactive MOPET is formed in the brain 1·5 hr after intraperitoneal injection of H^3-tyrosine.* In these experiments the amount of H^3-MOPET even exceeds that of H^3-MOPEG. The formation of labelled MOPET have been shown *in vitro* too, although very small amounts were formed by rabbit cortex slices[4] and by rat brain slices.[8]

In spite of the above mentioned evidence MOPET has not yet been shown to be present endogenously in the normal rat brain. Apparently no serious attempts have been performed to demonstrate its presence. In order to further elucidate this problem the following gas chromatographic procedure was adopted. This procedure detects even small amounts of free or conjugated MOPET in brain tissue.

Rats were killed by a blow on the head, and the brains (1·7–1·9 g) were rapidly removed and homogenized in 8·15 ml 0·4 N cold perchloric acid containing 10 mg sodium bisulphite and 4 mg EDTA. After centrifugation the clear supernatant was adjusted to pH 5·8 with 5 N potassium carbonate. Potassium perchlorate was precipitated at 0° for 30 min and then separated by centrifugation. The samples were adjusted to pH 6·0 with 1·0 ml 1·0 M acetate buffer, 200 μl glusulase (Endolab.) were added and the samples were incubated overnight (18–22 hr) at 37°. MOPET and some other neutral compounds were extracted with 3 × 12 ml ethyl acetate (nanograde, Mallinchrodt Chem.), 2·0 μg homovanillic acid methyl ester were added as reference compound and the solution was evaporated to dryness at room temperature under reduced pressure.

The almost invisible residue was transferred with 3 × 400 μl dry ethyl acetate to a small vial, the volume was reduced to approximately 200 μl with dry N_2 and the sample was reacted with 100 μl pentafluorpropionic anhydride (PFP, Pierce Chemical Co.) for 30 min at room temperature. The sample was reduced to dryness with dry N_2, redissolved in 200 μl dry ethyl acetate and 0·2–0·3 μl was injected in the gas chromatograph.

Conditions for gas chromatography; The column was six feet × 4 mm i.d. coiled glass, packed with 2·5 per cent OV–17 on Chromosorb G 80–100 mesh. The column temperature was maintained at 150° while the inlet, outlet and detector temperature was 180°. Ten per cent methane in argon was used as carrier gas. The polarization voltage of the Ni ^{63}-electroncapture detector was maintained at 50 V, pulse interval 50 μsec.

The gas chromatographic records on Fig. 1. show the presence in normal rat brain of a peak with exactly the same retention time as synthetic MOPET PFP-derivative ($t_{ret.\ sample}$ = 10·017 ± 0·014 min), (n = 10)†. ($t_{ret.\ MOPET}$ = 10·003 ± 0·016 min), (n = 8), the retention time of the reference homovanillic acid methyl ester derivative being 25 min under these conditions. Another derivative, using heptafluorbutyric anhydride instead of pentafluorpropionic anhydride gives identical conclusion ($t_{ret.\ MOPET}$ = 11·7 min under these conditions).

The above presented evidence for the presence of MOPET in rat brain tissue is further supported by thinlayer chromatography on cellulose powder (chloroform–acetic acid–water 2:2:1) or by paper chromatography (butanol–ethanol–water 4:1:1). Hydrolysed brain samples were chromatographed in these two systems, the powder and paper were eluted with methanol at the respective R_f-values for synthetic MOPET (R_f = 0·79 and 0·85 respectively), and the two extracts were prepared for gas

* M. Nielsen, L. Eplov and J. Scheel-Krüger in preparation.

† n = Number of different samples, analysed on different days. F. KAROUM *et. al.*, *Biochem. Med.* **5**, 505 (1971), presented additional gas chromatographic evidence for the presence of MOPET in rat brain.

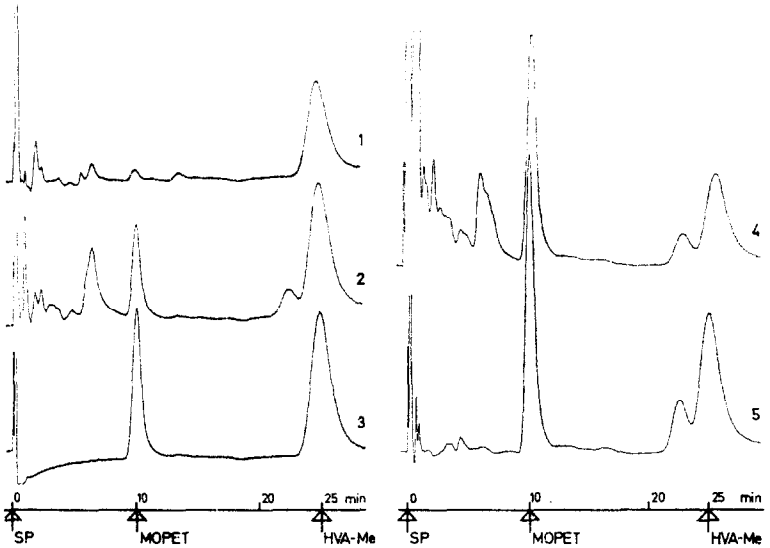

FIG. 1. Gas chromatographic records of; (1) One rat brain, processed as described in the text, but without glusulase added. (2) One rat brain, processed as described in the text. The peak at 10·017 min equivalents 108 ng MOPET per g. brain tissue, uncorrected for recovery. (3) PFP-derivative of 200 ng MOPET and 2·0 μg HVA–Me in 200 μl ethylacetate. (4) One rat brain plus 500 ng MOPET processed as described in the text. MOPET was added just before homogenization. Recovery of added MOPET approximates 85 per cent. (5) 500 ng MOPET in H_2O processed as described in the text, but without brain tissue added. Recovery approximates 90 per cent. SP, solvent peak. MOPET 3-methoxy-4-hydroxyphenylethanol. HVA–Me, homovanillic acid methyl ester used as reference compound.

chromatography. Both samples yielded peaks with the same retention time as synthetic MOPET.

It was found that the observed peak at 10·017 min on the gas chromatographic recording mainly appears after hydrolysing with Glusulase (Fig. 1). This is in agreement with previous results showing that C^{14} MOPET formed *in vivo* from C^{14} dopa appears as a strongly acidic conjugate.[6,7] M. Nielsen and coworkers found MOPET mainly as a conjugate in the H^3-tyrosine experiments mentioned above. The agreement is further extended to MOPEG, the alcohol formed from norepinephrine. This alcohol was found to be mainly conjugated in the normal rat brain too.[9]

The obtained results thus strongly indicate the presence of 3-methoxy-4-hydroxyphenylethanol, mainly as a conjugate, in the normal rat brain. Further identification and quantification is in progress.

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