Mass spectrometric identification of a new metabolite of pethidine

Many drugs containing phenyl groups undergo metabolic hydroxylation in the *para* position (Daily, 1971). However, as a route of metabolism of synthetic opiates, this pathway seems to have been reported only twice: in the biotransformation of methadone (Sullivan, Due & McMahon, 1972) and of (+)-propoxyphene (McMahon, Sullivan & others, 1973). Pethidine is extensively transformed in man to a number of metabolites (Mitchard, Kendall & Chan, 1972; cf. Asatoor, London & others, 1963), but according to the literature no metabolically formed hydroxypethidine has so far been found. By using combined gas chromatography-mass spectrometry we have now identified a pethidine metabolite, hydroxylated in the aromatic ring, in both rat and human urine.

Using the method of Knapp, Gaffney & McMahon (1972) to give a mixture of deuterium labelled and unlabelled drug we examined the artificial isotope pattern created in the mass spectra of the metabolites. In the present study we used pethidine labelled in the phenyl group with 5 deuterium atoms (pethidine-$^2H_5$) (Lindberg, Bogentoft & Danielsson, 1974).

Four male albino rats were injected intraperitoneally and two intravenously (tail vein) with an equimolar solution of pethidine hydrochloride and pethidine-$^2H_5$ hydrochloride (50 mg kg^{-1}), and the urine was collected overnight. Four of the samples (three treated i.p., one i.v.) were each treated in the following way. The pH of the urine (originally 5–6) was adjusted to 9 with 1M Na_2CO_3 and extracted with 15 ml of CH_2Cl_2. This was then back-extracted into 5 ml of 0·05M sulphuric acid and the pH readjusted to 9. Then pethidine and the basic metabolites were extracted into 5 ml of CH_2Cl_2.

In order to release conjugated metabolites, the urine from the remaining two rats was hydrolysed with β-glucuronidase containing sulphatase (Sigma Chemical Co) at pH 4 and 37° and then extracted as before after adjusting the pH to 9.

The organic solvent from each of the six samples was evaporated to dryness under a stream of nitrogen on a water-bath at 40°, and the residues were treated with 50 μl of a mixture of acetic anhydride–pyridine (1 : 1). After 15 min, excess reagent was removed by evaporation and the residues dissolved in 50 μl of methanol. Aliquots (1–4 μl) of the solutions were injected on an LKB 9000 gas chromatograph-mass spectrometer equipped with a glass column (length 6 ft) packed with 3% OV-17 on Gas-Chrom Q. The column temperature was kept at 200° for 5 min after injection of the sample and then increased to 225° at a rate of 5° min^{-1}.

Total ion current recordings of the extracts showed two major components, identified as pethidine and acetylated norpethidine by comparison of the retention times (3 and 14 min respectively) and mass spectra (cf. Lindberg & others, 1974) of authentic material. There was no significant difference in the chromatograms obtained after intraperitoneal and intravenous administration. The chromatograms also revealed the presence of a third component eluting after 12 min and, in the samples treated with β-glucuronidase, this consisted of 5–10% of the norpethidine content, whereas only minor amounts were detected in the untreated samples. Examination of the isotopic pattern in the mass spectrum of this component showed that substitution had occurred in the aromatic ring of pethidine, see Fig. 1A. The molecular ions at *m/e* 305 and 309, as well as the fragment ions, indicate that the peak is due to acetylated hydroxypethidine. This is further supported by comparison with synthetic *p*-hydroxypethidine* which upon acetylation showed identical gas chromatographic

* Prepared analogous to pethidine-$^2H_4$ (Lindberg & others, 1974) by condensing 4-methoxybenzyl cyanide and *N*-methyl-bis(2-chloroethyl)amine and cleaving the ether with 48% HBr in water.

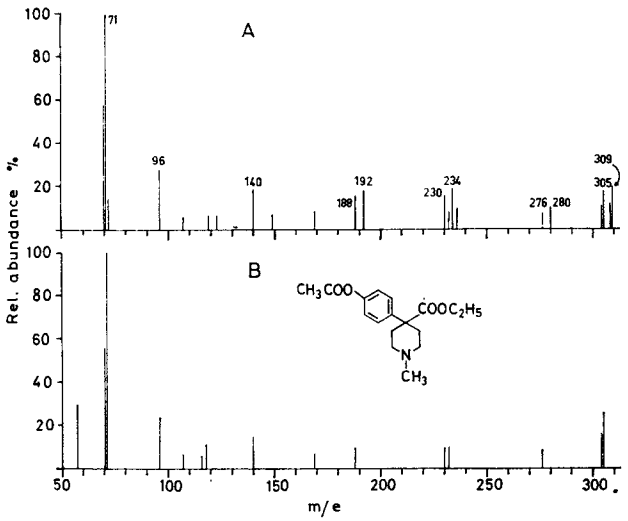

FIG. 1A. Mass spectrum of the acetylated metabolite from rat, given pethidine and pethidine-$^3H_5$ (from β-glucuronidase treated urine).
B. Mass spectrum of acetylated synthetic *p*-hydroxypethidine.

and mass spectrometric properties, see Fig. 1B. The assignment of the hydroxyl group to the *para* position is somewhat tentative and partly based on analogy with the well-known *p*-hydroxylation of other compounds.

In order to make a preliminary investigation of the significance of pethidine hydroxylation in man, a healthy male volunteer was given pethidine (100 mg orally of the labelled mixture used in the animal experiments) and approximately 500 ml of urine was collected during the next 10 h. After β-glucuronidase - sulphatase treatment and extraction with 500 ml of CH_2Cl_2, the extract was concentrated to 15 ml and analysed as described above. G.c.–m.s. analysis showed the presence of hydroxypethidine in the urine. In this experiment, hydroxypethidine appeared as a minor metabolite amounting to less than 1% of the norpethidine content.

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REFERENCES

ASATOOR, A. M., LONDON, D. R., MILNE, M. D. & SIMENHOFF, M. L. (1963). *Br. J. Pharmac.*, **20**, 285–298.

DALY, J. (1971). *Handb. Exp. Pharmac.*, Vol. **28**, 285–311.

KNAPP, D. R., GAFFNEY, T. E. & MCMAHON, R. E. (1972). *Biochem. Pharmac.*, **21**, 425–429.

LINDBERG, C., BOGENTOFT, C. & DANIELSSON, B. (1974). *Acta pharm. suecica*, **11**, 201–210.

MCMAHON, R. E., SULLIVAN, H. R., DUE, S. L. & MARSHALL, F. J. (1973). *Life Sci.*, **12**, part 2, 463–473.

MITCHARD, M., KENDALL, M. J. & CHAN, K. (1972). *J. Pharm. Pharmac.*, **24**, 915.

SULLIVAN, H. R., DUE, S. L. & MCMAHON, R. E. (1972). *J. Am. chem. Soc.*, **94**, 4050–4051.