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SIMULTANEOUS DETERMINATION OF MEPERIDINE AND NORMEPERIDINE IN BIOFLUIDS

HAZEL H. SZETO* and CHARLES E. INTURRISI**

Department of Pharmacology, Cornell University Medical College, New York, N.Y. 10021 (U.S.A.)

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SUMMARY

A method employing solvent extraction and gas–liquid chromatography has been developed for the simultaneous determination of meperidine and its N-demethylated metabolite, normeperidine, in biofluids. Normeperidine is analyzed as the heptafluorobutyryl derivative. Using a flame ionization detector, the lower limit of sensitivity of the method is 0.02 μg/ml of biofluid for both compounds.

Samples of plasma obtained from obstetrical patients, following a single therapeutic dose, were found to contain higher levels of meperidine than concurrent samples of amniotic fluid. Normeperidine could not be detected in either biofluid after a single dose. There is, however, a gradual accumulation of normeperidine in plasma after repeated doses as determined in samples from cancer patients.

The method can also be used to determine the disposition of meperidine and the accumulation of normeperidine in the cat.

INTRODUCTION

Meperidine is a narcotic analgesic that is widely used for the relief of pain. In man, the major metabolic pathways of meperidine involve hydrolysis to form meperidinic acid, and N-demethylation to normeperidine, which is followed by hydrolysis to normeperidinic acid[1-4]. Normeperidine is the only metabolite that has been shown to possess significant pharmacologic activity[5] (Fig. 1). Animal studies have shown that the N-demethylated metabolite is half as potent as meperidine as an analgesic, but twice as potent as a convulsant[6,7].

Normeperidine has not been reported in human plasma after a single dose of meperidine. The purpose of this report is to describe a specific and sensitive method for the simultaneous determination of meperidine and normeperidine in biofluids.
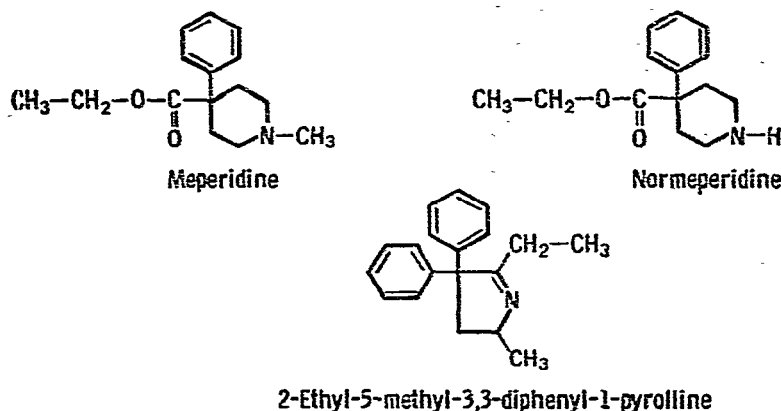
2-Ethyl-5-methyl-3,3-diphenyl-1-pyrolline

Fig. 1. Structural formulae of meperidine, normeperidine and the internal standard.

MATERIALS AND METHODS

Chemicals and reagents

Meperidine hydrochloride and normeperidine hydrochloride were generously supplied by Dr. F. Nachod of the Sterling-Winthrop Research Institute (Rensselaer, N.Y., U.S.A.). The internal standard, 2-ethyl-5-methyl-3,3 diphenyl-1-pyrolline hydrochloride, was provided by Dr. H. R. Sullivan of the Lilly Research Labs. (Indianapolis, Ind., U.S.A.) (Fig. 1).

The n-hexane and ethyl acetate are spectral grade, all other solvents are reagent grade. Anhydrous ethyl ether is obtained from Fisher (Pittsburgh, Pa., U.S.A.).

Stock solutions

Aqueous solutions of meperidine, normeperidine and the internal standard, each at a concentration of $4 \mu g/ml$, are prepared and kept refrigerated.

Sample extraction from plasma

The method for extraction is adapted from that described by Inturrisi and Verebely[8] for the extraction of methadone from plasma.

To plasma (0.5—2.0 ml) in a 15-ml siliconized centrifuge tube with a PTFE-lined screw cap is added 0.15 ml of the solution of internal standard, 0.25 ml of 2.5 N sodium hydroxide and 2 drops of octyl alcohol. The sample is extracted with 5 ml of anhydrous ethyl ether by shaking for 5 min in an automatic shaker and centrifuged for 5 min at 500 g. The ethyl ether layer (upper) is carefully removed and saved. The extraction is repeated again with 5 ml of ethyl ether. The ether layers are combined and extracted with 5 ml of 0.2 N hydrochloric acid by shaking for 10 min and followed by centrifugation for 5 min. The ether phase (upper) is aspirated and discarded. The acid phase is then washed with 5 ml of hexane by shaking for 5 min and centrifuged for 3 min. The hexane wash (upper phase) is discarded. The washed acid phase is made alkaline by addition of 3 drops of 50% sodium hydroxide (pH >11), and extracted by shaking with 7 ml of ether for 10 min. After centrifugation for 5 min, the ether phase is carefully transferred to a 12-ml siliconized centrifuge tube and evaporated to dryness in a waterbath at 42°.

Sample extraction from amniotic fluid

Same procedure as described above for the extraction from plasma.

Preparation of the heptafluorobutyryl (HFB) derivative of normeperidine

A 3 % solution (40 μl) of heptafluorobutyrylimidazole (HFBI) in ethyl acetate is added to the final dry extract, mixed and allowed to sit at room temperature in the dark for 30 min. The mixture is then dried by a gentle stream of nitrogen. The sample is reconstituted with 30 μl of cyclohexane by warming at 40°, and 1–2 μl is injected into the gas chromatograph.

Gas–liquid chromatography (GLC)

The GLC analysis is performed on a Varian Aerograph Model 2700 equipped with a flame ionization detector. The column is a 6-ft. long glass spiral with a 2-mm I.D. The packing material consists of 3 % OV-17 on 80–100 mesh Gas-Chrom Q. The temperatures of the detector and the injection port are 275° and 250°, respectively. The carrier gas is helium at a flow-rate of 34 ml/min. Hydrogen and air flow are adjusted to give maximal detector response. Hydrogen flow-rate is 30–33 ml/min, and air flow-rate is 300–350 ml/min. The oven temperature is 175°. Detector sensitivity is varied from 4×10^{-11} to 16×10^{-11} A/mV at full scale as required.

Quantitation and calibration curves

Quantitation is performed by drawing in a baseline and measuring the peak height from the midpoint of the baseline. Calibration curves are constructed by plotting the ratio of the peak height of the compound to that of the internal standard against the concentration added. Standard calibration curves are obtained by adding meperidine and normeperidine in selected amounts from 0.04–1.0 μg to 2.0 ml of plasma or amniotic fluid and extracting according to the above procedure. The amount of each compound in a sample is determined by converting the peak height ratio into the absolute amount of compound present. Linearity of the standard curves in the range from 0.04–1.0 μg for both compounds allows for such conversion. The lower limit of sensitivity is defined as the lowest point on the calibration curve which gives at least a 5 unit deflection above baseline at attenuation 4×10^{-11}. This was found to be 0.02 μg/ml for both compounds in plasma and amniotic fluid.

RESULTS AND DISCUSSION

Many of the older methods for the determination of meperidine and normeperidine in human biofluids are based on spectrophotometric measurements of methylorange complexes[1–4,7]. These methods have been shown to be non-specific[9] and limited in sensitivity. Both of these considerations are critical in any method to be employed in pharmacokinetic studies. A fluorometric method for meperidine as described by Dal Cortivo et al.[10] has a lower limit of sensitivity of approx. 0.3 μg/ml. Recently several GLC methods have been reported for the quantitation of meperidine. Goehl and Davison[11] reported a GLC method for meperidine with a lower limit of sensitivity of 0.1 μg/ml. In 1974, Stambaugh and Wainer[12] published a method for quantitating 0.01 μg/ml of meperidine. These methods, however, do not also measure normeperidine levels. Klotz et al.[13] developed a method for both meperidine and nor-

meperidine by analyzing normeperidine as the trifluoroacetyl derivative, with a lower limit of sensitivity of 0.1 μg/ml. Chan *et al.*[14] reported a method for the simultaneous determination of meperidine and normeperidine using Carbowax as the stationary phase, which claimed to be capable of detecting as little as 0.02 μg/ml of both compounds in a 5-ml sample. However, we were not able to reproduce their method in our laboratory. The method we have developed supersedes most available methods both in sensitivity and by providing simultaneous quantitation of meperidine and normeperidine.

The conditions for the extraction of meperidine and normeperidine from biofluids were modified from the standard procedure for basic amines[8] to favor the recovery of both compounds. For example, we found that while *n*-butyl chloride would provide a good recovery of meperidine, a more polar solvent such as ethyl ether was necessary for optimal recovery of normeperidine.

Due to the close similarity in the chemical structure of meperidine and normeperidine, a commonly used non-polar phase such as SE-30 was not sufficient to separate the two compounds over a wide range of temperature conditions. The relatively more polar phases such as QF-1 and OV-17 allowed baseline separation, but did cause a moderate amount of tailing of the normeperidine. It was also found that although the linearity of the calibration curve for meperidine did extend down to 0.02 μg/ml, the linear range for normeperidine could be extended only to 0.08 μg/ml.

In order to overcome the problem of adsorption of normeperidine onto the packing material, it was necessary to form a derivative. A HFB derivative was chosen because it can be formed readily from normeperidine and results in a derivative with much improved column characteristics under the GLC conditions as described. Also, although not utilized at this time, the HFB derivative could be analyzed by an electron capture detector with a further increase in sensitivity.

Heptafluorobutyryl derivatives may be prepared from heptafluorobutyryl-imidazole (HFBI) or heptafluorobutyrylanhydride (HFBA). HFBI is preferred over HFBA because it does not release acid into the reaction mixture, an inert base imidazole is produced instead. The derivative procedure is adapted from a general method described by Franken and Trijbels[15] for aliphatic amines. Conversion of normeperidine to the HFB derivative was found to be complete in 30 min at room temperature. And in agreement with the above paper, it was essential that the reaction be carried out in the dark, otherwise a greenish yellow color results and the conversion is not reproducible. It was found that most of the excess reagent could be removed by drying with nitrogen. Cyclohexane was chosen as the solvent to reconstitute the compounds for GLC because it did not dissolve any of the more polar by-products, and thus produced chromatograms without any interfering peaks. With the preparation of the HFB derivative, it was possible to extend the linearity of the calibration curve of normeperidine down to 0.02 μg/ml. The method as described can routinely be used to measure as little as 0.02 μg/ml of meperidine and normeperidine with an initial sample volume of 1 ml of biofluid. This high degree of sensitivity enables us to collect relatively small biofluid samples for pharmacokinetic studies.

Examples of chromatograms obtained under the conditions described above are given in Fig. 2. The multi-step extraction procedure results in an extract that is free of interfering peaks. In most cases it was possible to introduce samples into the gas chromatograph every 8–10 min.
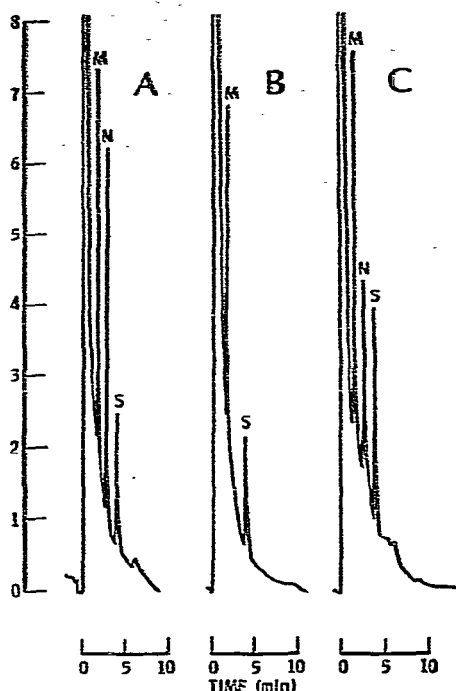
Fig. 2. Chromatograms of human plasma extracts. Retention times: meperidine (M), 1.8 min; normeperidine (N), 2.8 min; internal standard (S), 4.2 min. (A), Extract of control plasma to which was added 0.40 μg of meperidine (M), 0.40 μg of normeperidine (N), and 0.40 μg of internal standard. (B), Extract of plasma from an obstetric patient who received a single dose of meperidine, 1C0 mg i.m. (C), Extract of plasma from a cancer patient who received 10 doses of meperidine, 75 mg i.m. Detector sensitivity was 8×10^{-11} A/mV at full scale.

Fig. 2A shows the chromatogram obtained from an extract of control plasma to which was added 0.40 μg of meperidine, 0.40 μg of normeperidine, and 0.40 μg of internal standard. The retention times are: 1.8, 2.8, and 4.2 min, respectively.

Fig. 2B shows the chromatogram obtained from an extract of the plasma from an obstetrical patient who had received a single dose of meperidine, 100 mg intramuscular (i.m.). The sample was collected 30 min after the administration of the dose. The internal standard was added to 1.0 ml of sample plasma and the extract prepared as described in Materials and Methods. As can be seen in Fig. 2B the extract contained meperidine, but no normeperidine was detected.

Fig. 2C shows the chromatogram of an extract of a 0.5-ml plasma sample from a cancer patient who had received a total of 10 doses of meperidine, 75 mg i.m. The sample was collected 1 h after the last dose. It can be seen that the sample contained both meperidine and normeperidine.

The method was employed in the determination of the plasma and amniotic fluid concentration of meperidine and normeperidine in obstetrical patients after a single dose of meperidine. Amniotic fluid was obtained by amniocentesis at various times after the administration of the dose, and a blood sample was collected at the same time. The results in Table I show that meperidine is present in amniotic fluid

TABLE I

PLASMA AND AMNIOTIC FLUID LEVELS OF MEPERIDINE IN OBSTETRICAL PATIENTS AFTER A SINGLE 100-mg I.M. DOSE

Subject	Weeks of gestation	Time after dose (min)	Meperidine level ($\mu g/ml$)	
			Plasma	Amniotic fluid
1	20	15	0.34	0.00
2	20	30	0.32	0.02
3	19	60	0.37	0.04
4	20	120	0.12	0.07

within 30 min after intramuscular administration, and the concentration remains lower than that in plasma up to 3 h after the dose. Normeperidine did not attain detectable levels in plasma or amniotic fluid during that time period.

The method has been used to compare the plasma levels of meperidine and normeperidine in samples from obstetrical patients given a single dose of meperidine with the plasma levels from cancer patients given multiple doses. Table II shows the data obtained from some of these patients. All samples were collected 1 h after the close.

TABLE II

PLASMA LEVELS OF MEPERIDINE AND NORMEPERIDINE IN PATIENTS RECEIVING SINGLE AND MULTIPLE I.M. DOSES OF MEPERIDINE

Subjects 1–4 are obstetrical patients and subjects 5–8 are cancer patients.

Subject	Dose (mg)	Number of doses	Time after dose (h)	Plasma level ($\mu g/ml$)	
				Meperidine	Normeperidine
1	100	1	1	0.29	0.00
2	100	1	1	0.37	0.00
3	100	1	3	0.07	0.00
4	100	1	3.5	0.11	0.00
5	100	9	1	0.54	0.28
6	75	10	1	0.38	0.18
7	75	5	1	0.36	0.13
8	100	70	4	0.16	0.48

The data indicate that the peak plasma level of meperidine at 1 h after dosing was, on the average, somewhat higher in those patients given multiple doses compared to those given a single dose. The half-life of meperidine in normal volunteers after a single intravenous dose has been reported by Klotz et al.[13] and Mather et al.[16] to be 3.2 h and 3.7 h, respectively. Therefore, since the dosing interval in the cancer patients was 4–6 h, some accumulation of plasma meperidine could be expected. In agreement with the results of Klotz et al.[13] no normeperidine was detected in plasma up to 3.5 h after a single dose. However, we do find relatively high levels of normeperidine in the plasma of patients who have received multiple doses of the drug. The half-life of normeperidine in man is not known. It appears that there is an accumulation of the N-demethylated metabolite in plasma after repetitive doses, suggesting that the half-

life of the metabolite is longer than that of the parent drug. This finding may have considerable clinical significance since normeperidine has been shown in animal studies to be 2-3 times as toxic as meperidine[5]. Meperidine and normeperidine produce both excitatory and depressant effects with the excitatory effects predominant for normeperidine. In animals toxic doses of normeperidine led to convulsions with varying degrees of respiratory depression, and eventually death[6,7]. Although the depressant effects can be completely antagonized by a narcotic antagonist such as naloxone, Gilbert and Martin[17] have shown, in mice, that naloxone can only partially antagonize the convulsions produced by normeperidine.

This method was also used to study the disposition of meperidine and normeperidine in the plasma of a cat who received 4 doses of meperidine. Meperidine hydrochloride (5 mg/kg, i.m.) was injected at 4-h intervals and blood samples were collected at various times from a chronic indwelling catheter. The results are shown in Table III.

It can be seen that meperidine is rapidly absorbed after i.m. administration, and the levels fall rapidly during the first hour. Normeperidine was not detected in plasma until 3 h after the first dose. With each succeeding dose, the meperidine levels decline rapidly between the 1/4- and the 4-h samples. In contrast there is a gradual accumulation of normeperidine in the plasma following each dose of meperidine. Thus the ratio of normeperidine/meperidine at 4 h after dosing is increased from 0.26 after the first dose to 0.76 after the fourth dose.

TABLE III

PLASMA LEVELS OF MEPERIDINE AND NORMEPERIDINE IN A CAT FOLLOWING EACH OF 4 DOSES OF MEPERIDINE (5 mg/kg I.M.)

The dose was repeated at 4-h intervals.

Dose	Time after dose (h)	Plasma level (μg/ml)	
		Meperidine	Normeperidine
1st	1/12	6.02	0.00
	1/4	2.40	0.00
	1/2	2.00	0.00
	1	0.95	0.00
	2	0.74	0.00
	3	0.37	0.06
	4	0.39	0.10
2nd	1/4	3.87	0.13
	4	0.51	0.32
3rd	1/4	4.39	0.25
	4	0.78	0.36
4th	1/4	5.08	0.49
	4	0.93	0.71

These results from the cat are similar to those that we have found in preliminary studies in human subjects. The data indicate that N-demethylation is a major pathway of biotransformation of meperidine in both cat and man. Also both cat and man show an accumulation of normeperidine when given meperidine in repeated doses at the usual therapeutic dosing interval.

The development of a specific and sensitive method for the quantitation of meperidine and normeperidine will now permit us to study the pharmacokinetics of meperidine and normeperidine in man and in an animal model, the cat.

ACKNOWLEDGEMENTS

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