Determination of Mepivacaine in Blood and Urine

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A gas chromatographic procedure for the quantitative determination of mepivacaine (Carbocaine) in blood and urine is described. Using a 5.0-ml sample, the procedure can detect 0.5 μg of mepivacaine hydrochloride per ml of blood or urine. Recoveries of mepivacaine from aqueous, blood and urine samples were 85–96 per cent, 83–92 per cent and 85–94 per cent, respectively. Application of this procedure to the quantitative determination of mepivacaine in blood and urine samples of patients receiving 2 per cent mepivacaine hydrochloride prior to delivery is described.

CONSIDERABLE interest in the pharmacology of mepivacaine (Carbocaine) has resulted in the development of several[1-5] progressively more elegant procedures for its quantitative determination in biological fluids. Of these, the gas chromatographic procedure is the most specific and sensitive. Using this technique, Pratt *et al.*[6] developed a method which recovered 56 per cent of the drug present in blood specimens. To improve recovery, the protein precipitation in Pratt's procedure was replaced by direct extraction of the sample. The application of this modification to blood and urine samples is described. In addition, two metabolites of mepivacaine were isolated and identified.

Material and Method

APPARATUS

A Barber Colman series 5000 gas chromatograph equipped with a flame ionization detector and a Honeywell 1 mv recorder containing a Disc-integrator was used. A four-foot glass column, ¼ inch i.d., containing 3 per cent OV-1 on 100/120 mesh gas chrom Q was conditioned at 260 C for 24 hours under nitrogen flow prior to use and periodically thereafter. Operating temperatures were: column, 230 C; injection port, 250 C; detector, 260 C. The carrier gas was nitrogen, at a flow rate of 65 ml/min at 40 psi valve pressure. Hydrogen and air were adjusted to give maximum sensitivity. Recorder speed was 12 inches per hour.

ANALYTICAL METHOD

Whole blood (5 ml) or urine (10 ml) was transferred through a pipette into a glass-stoppered bottle or a separatory funnel. Using indicator paper to check, we added 6 N NaOH until the *p*H was 12. The alkaline solution was extracted with ten times its volume of chloroform (analytical reagent grade, Mallinckrodt) by shaking vigorously for five to ten minutes. After the phases separated, the upper layer was removed and discarded. The chloroform was filtered through a plug of glass wool covered with approximately 0.3 g of anhydrous sodium sulfate. An aliquot (usually 80 per cent) of the extract was collected, transferred to an evaporating dish, and evaporated to dryness at room temperature. After rinsing the sides of the evaporating dish with 1 ml of acetone, solution was transferred quantitatively to a 1-ml conical polyethylene tube.§ This solution was concentrated to approximately 0.1 ml by evaporation under a fine stream of nitrogen. The rinsing and evaporation step was repeated four times. After the fourth rinse the solution was evaporated to dryness. The residue was redissolved in 0.05 ml of a solution containing the internal standard (50 mg chlorcyclizine hydrochloride in 100 ml acetone). Immediately, a 5-μl aliquot was injected into the gas chromatograph.

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§ #2991-B35, A. H. Thomas Co., Philadelphia, Pennsylvania.

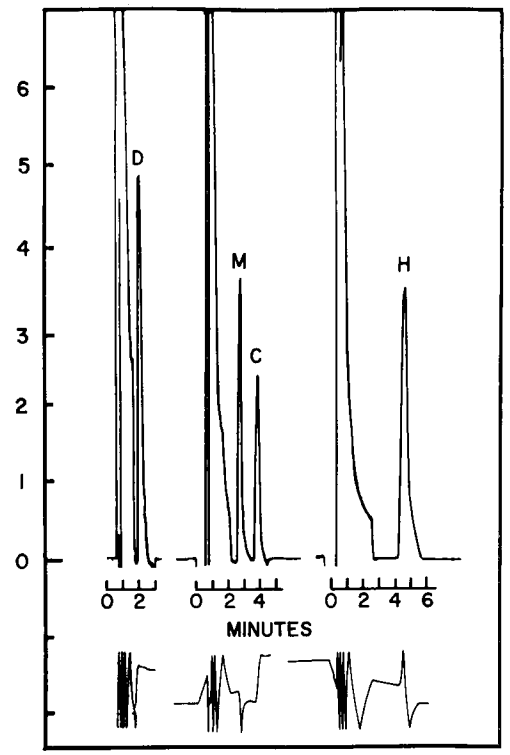

FIG. 1. Gas chromatograms of N-desmethylmepivacaine (D), mepivacaine (M), chlorocyclizine (C) and 4-hydroxymepivacaine (H).

During each series of analyses 5-μl injections of each of the following control samples must be made: 1) 50 mg mepivacaine in 100 ml acetone (known control); 2) the internal standard; 3) an extract, obtained as described above, of a solution of known concentration of mepivacaine in 100 ml of sample (0.2 mg for blood and 1.0 mg for urine). Similar 5-μl injections of solutions of N-desmethylmepavicaine and 4-hydroxymepivacaine were made. (The solutions of N-desmethylmepivacaine and 4-hydroxymepivacaine were made by dissolving 100 mg of each compound in 5 ml water and diluting to 100 ml with acetone.)

CLINICAL MATERIAL

Sixty-seven patients receiving peridural caudal anesthesia for labor and delivery were premedicated with demerol or with demerol and an ataractic agent. Divinyl catheters were inserted in the caudal canal prior to induction of anesthesia. Through these catheters test doses of 7 ml of 2 per cent mepivacaine hydrochloride (Carbocaine) were injected. If the patient's lower extremities showed signs of partial anesthesia, it was assumed that the catheter was inserted properly, and the remainder of the anesthetic dose, usually 18–20 ml of 2 per cent mepivacaine hydrochloride, was administered. After 60–90 minutes any patient who indicated a need for more anesthesia was given another injection of 10 ml of 2 per cent mepivacaine hydrochloride.

Results

The relative retention times for mepivacaine hydrochloride, chlorcyclizine hydrochloride, N-desmethylmepivacaine and 4-hydroxymepivacaine were 0.75, 1.00 (4 min), 0.63 and 1.25, respectively. Gas chromatograms of the substances are shown in figure 1. The lowest concentration of mepivacaine that could be detected was 0.04 μg/μl of acetone. This corresponds to the amount of mepivacaine extracted from 5 ml of sample containing 0.05 mg of mepivacaine per 100-ml sample.

To ascertain the accuracy of the gas chromatographic procedure, recovery experiments were carried out with solutions of mepivacaine added to water, blood and urine (table 1). Forty-five minutes after the administration of peridural caudal anesthetic doses of 500–560 mg of mepivacaine, the concentrations in the blood of 67 patients ranged from 0.05 to 0.3 mg/100 ml blood. Analyses of aliquots of urine obtained within 1–2 hours after administration of the drug indicated that mepivacaine concentrations ranged from 0.05 to 2.2 mg/

TABLE 1. Recovery of Mepivacaine Hydrochloride from Aqueous, Blood and Urine Solutions of Known Concentration

Solution	Concentration of Mepivacaine Hydrochloride (mg/100 ml)	Number of Determinations	Per Cent Recovery
Aqueous	5.0	5	96 ± 3
	1.0	5	92 ± 2
	0.5	5	85 ± 4
Blood	5.0	5	92 ± 2
	1.0	5	89 ± 2
	0.5	5	83 ± 4
Urine	5.0	5	94 ± 2
	1.0	5	89 ± 1
	0.5	5	85 ± 4

100 ml urine. Figure 2 presents a gas chromatogram of the extract of the urine of a patient who received an anesthetic dose of mepivacaine. Three of the four peaks were identified as mepivacaine (A), chlorcyclizine (B), and 4-hydroxymepivacaine (C). The fourth peak was not identified. Identification was based on direct comparison of the gas and thin-layer chromatograms of the isolated compounds with those of the authentic products.

Calculation of the concentration of mepivacaine (M) in biological specimens using chlorcyclizine hydrochloride as an internal standard is as follows:

$$M(\text{mg}/100\text{ ml}) = \left(\frac{125}{40}\right)\left(\frac{1}{V_s}\right)\left(\frac{D_s^c}{D_s^m}\right)\left(\frac{D_e^m}{D_e^c}\right)$$

D_e^m and D_e^c are the disc integrator readings for the hydrochlorides of mepivacaine and chlorcyclizine, respectively, in the extract. D_s^c and D_s^m are the disc integrator readings for the 5-μl aliquots of the solutions of chlorcyclizine hydrochloride (internal standard) and mepivacaine hydrochloride (known) reference solutions, respectively. V_s is the volume of sample taken for analysis.

Discussion

The modified gas chromatographic procedure devised for this study indicates that a higher percentage of mepivacaine is recovered by direct extraction of blood than by extraction following deproteinization. Keenaghan [7] came to the same conclusion about lidocaine (Xylocaine®) and prilocaine (Citanest®). In addition, the extract can be assayed quantitatively by gas chromatography This procedure is simple, more specific and more sensitive than the colorimetric procedures involving methyl orange and cis-aconitic anhydride. Tertiary amines would interfere with the cis-aconitic anhydride procedures and any organic base would interfere with the methyl-orange method. Metabolites of mepivacaine, N-desmethyl- and 4-hydroxymepivacaine were identified by gas and thin-layer chromatography.

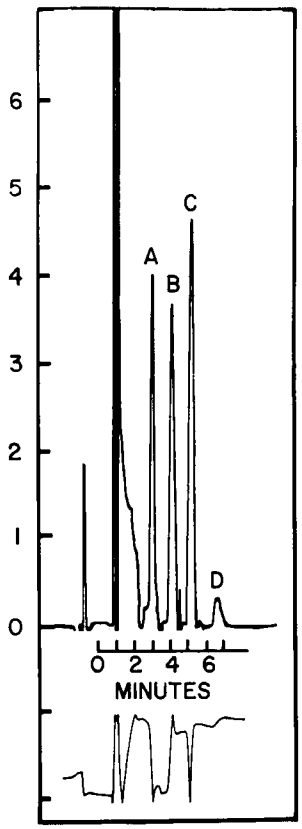

FIG. 2. Gas chromatogram of the extract of an aliquot of the urine from a patient who received 25 ml of 2 per cent mepivacaine hydrochloride. Identifiable substances are mepivacaine (A), chlorcyclizine (B) and 4-hydroxymepivacaine (C). Peak D was not identified.

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