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Urinary Metabolites of Chloroprocaine Studied by Combined Gas Chromatography–Mass Spectrometry

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The purpose of this study was to identify the metabolic pathway of 2-chloro-4-aminobenzoic acid (CABA), a primary metabolite of chloroprocaine. Urine was collected from 4 healthy, pregnant women following the epidural administration of 600 mg chloroprocaine. The urinary metabolites were extracted with ether and identified and quantitatively estimated with a gas chromatography–mass spectrometry instrument.

The analysis showed that CABA is converted to N-acetyl-CABA and subsequently excreted in the urine. (Key words: Anesthetics, local: chloroprocaine. Measurement techniques: gas chromatography; mass spectrometry. Metabolism: metabolites.)

CHLOROPROCAINE is an ester-type local anesthetic agent which is rapidly hydrolyzed by plasma pseudocholinesterase, yielding the primary metabolites, diethylaminoethanol and 2-chloro-4-aminobenzoic acid (CABA). The latter has been assumed to be eliminated as a conjugate,[1] but the structure of this metabolite has not yet been identified. Chloroprocaine is used extensively in providing obstetric analgesia and anesthesia, and for this reason, it was desirable to identify the metabolic pathways by which chloroprocaine and CABA are eliminated.

Materials and Methods

Chloroprocaine was obtained from Pennwalt Corp. (New York, U. S. A.); 2-chloro-4-aminobenzoic acid (CABA) from Sigma Chemical Company (St. Louis, U. S. A.); Para-aminosalicylic acid (PAS) from Apothekernes Laoboratorium (Oslo, Norway); Nitrosomethylurea from K and K Laboratories (California, U. S. A.); and the stationary phase (OV 17) and solid support (Gas Chrom Q®) were obtained from Applied Science Laboratories (Pennsylvania, U. S. A.). N-acetyl-CABA was prepared by heating CABA with a mixture of acetic anhydride and acetic acid (1:1), followed by extraction of the product into diethyl ether.

Urine samples were collected from 4 healthy, pregnant women through an indwelling urinary catheter, after the administration of 600 mg 3 per cent chloroprocaine in the epidural space. The patients were all scheduled for elective cesarean section and had received no medication prior to surgery apart from an antacid consisting of a mixture of aluminium and magnesium hydroxide. Urine samples were collected at the time of delivery and two hours later, corresponding to about one and three hours after the epidural injection, respectively. The urine was immediately placed on ice and subsequently stored at −70° C before analysis.

The organic acids were extracted three times from aliquots of acidified (*p*H 1) urine samples (5 ml) using diethylether (15 ml × 3) as solvent. The combined extracts were dried over anhydrous sodium sulfate, methylated with diazomethane liberated from N-nitrosomethylurea, and concentrated to a small volume (0.3 ml) in a stream of nitrogen before injection into the gas chromatography–mass spectrometry (GC–MS) instrument. This consisted of a Varian® 1440 gas chromatograph, a molecular separator of the glass frit type, and a single focusing mass spectrometer type CH 7 (Varian® MAT, Bremen, W. Germany), operated at an ionizing energy of 70 eV. The gas chromatograph was equipped with a packed glass column (2 m × 1/4 in o.d.) filled with 10 per cent OV 17 on Gas Chrom Q®, 80/100 mesh. Helium was used as a carrier gas (30 ml/min). The GC–MS was connected on line to a computer system, Spectro System 100 MS (Varian MAT, Bremen, W. Germany).

For quantitative analysis of the metabolites, para-aminosalicylic acid (PAS) was added as an internal standard to the urine samples prior to the ether extraction procedure. The relative responses of PAS and the metabolites were determined by adding

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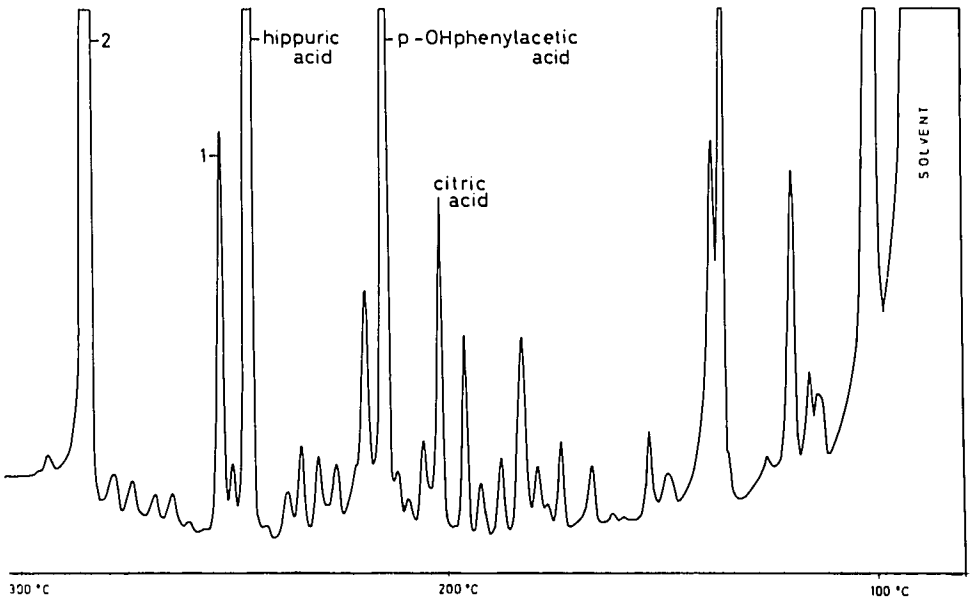

FIG. 1. Gas chromatogram of the organic acids in the urine of a patient given chloroprocaine, epidurally. The drug (600 mg) was administered three hours prior to collection of the urine sample. The organic acids were extracted by diethyl ether and converted into methyl esters before gas chromatography on a GC-column containing 10 per cent OV 17 on Gas Chrom Q, as described in the text. The temperature was programmed from 80–300° C at a rate of 8° C/min. Peaks 1 and 2 are absent in urine from normal females receiving no medication.

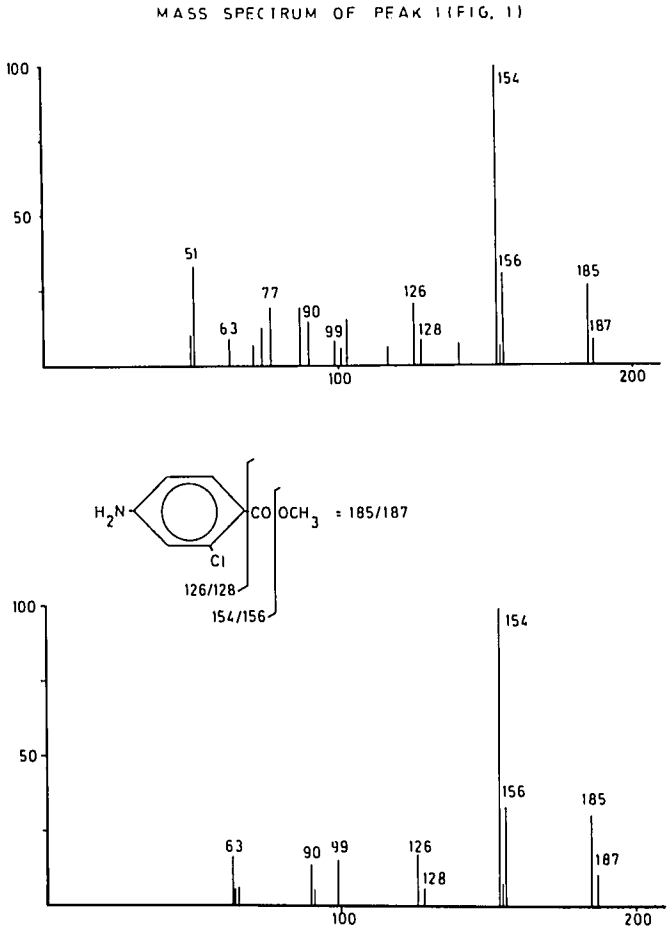

FIG. 2. Mass spectra of peak 1 of figure 1 (top) and authentic CABA (bottom). The spectra were recorded in a combined GC–MS instrument as described in the text.

known amounts of PAS, CABA, and N-acetyl-CABA to normal urine, which then were subjected to ether extraction, methylation, and analysis by GC–MS. Peak areas rather than heights were used for calculation of the results.

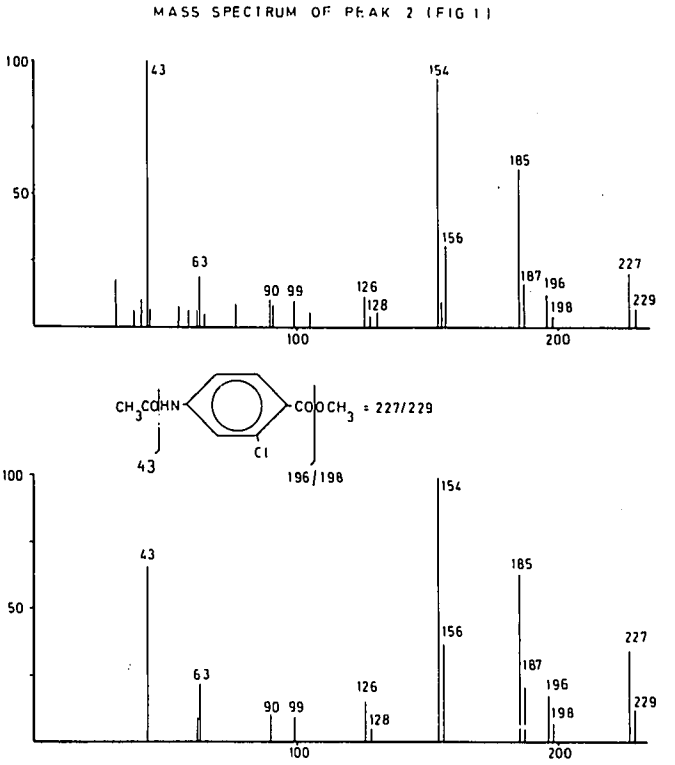

FIG. 3. Mass spectra of peak 2 of figure 1 (top) and of synthetic N-acetyl-CABA (bottom).

Results

Figure 1 shows the gas chromatogram of the methylated organic acids in a urine sample collected three hours after administration of 600 mg chloroprocaine for epidural anesthesia. The mass spectrum of peak 1 in the gas chromatogram is shown in figure 2 (top). This is in principle identical to the mass spectrum of authentic CABA (fig. 2, bottom). Also the GC-retention times of peak 1 and authentic CABA were identical. Peak 1 (fig. 1) can therefore be identified as unmetabolized CABA.

The mass spectrum of the dominating peak 2 is shown in figure 3 (top). A chlorine-containing molecular ion appeared at m/e 227/229 and the fragment ions m/e 196/198 were due to loss of a methoxy group. (The dual molecular numbers cited above reflect the presence of ^{35}Cl and ^{37}Cl isotopes in the fragments.) The presence of a large fragment ion at m/e 43 indicated occurrence of an acetyl group. The remaining fragments in the spectrum were almost identical to the fragmentation pattern of CABA, suggesting that the structure of peak 2 might be N-acetyl-CABA. This compound was subsequently synthesized by acetylation of authentic CABA. The mass spectrum of synthetic N-acetyl-CABA was identical to that of peak 2 (fig. 3, bottom), and moreover, the two compounds had identical retention times on two different GC-columns (OV 17, SE 30). This shows that the identity of peak 2 is N-acetyl-CABA, and from the chromatogram (fig. 1), it is evident that this is the dominating metabolite in the urine after chloroprocaine administration.

Organic acids in the body are often conjugated with glycine before excretion. Attempts were made to identify such a CABA-glycine conjugated, using the computerized GC–MS system and searching for characteristic fragments of this compound (molecular ion, molecular ion minus a methoxy group, minus a methyl ester group, etc.). No indications were found for the presence of a glycine conjugate.

TABLE 1. Amounts of CABA and N-acetyl-CABA in the Urine after Administration of Chloroprocaine (600 mg, Epidurally)

Patient	Hours After Injection	CABA (mg/l)	N-acetyl-CABA (mg/l)	Approximate Ratio (N-acetyl-CABA:CABA)
1	1	14,2	17,1	1:1
	3	16,3	94,1	6:1
2	1	Urine not collected due to wrongly placed Foley catheter		
	3	14,7	110,9	7:1
3	1	7,5	17,8	2:1
	3	14,0	92,6	7:1
4	1	10,7	21,2	2:1
	3	10,1	106,0	10:1

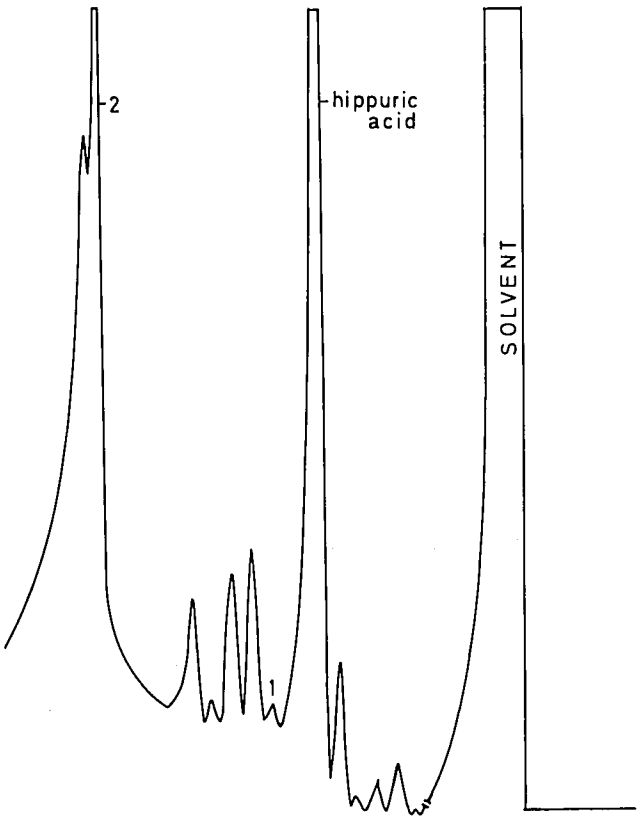

FIG. 4. Gas chromatogram of the organic acids in the urine of a newborn baby. The mother had received 600 mg chloroprocaine prior to the birth. Experimental conditions were the same as those in figure 1, except that the temperature was programmed from 150°–300° C at a rate of 8° C/min. Peak 1 = CABA; Peak 2 = N-acetyl-CABA. (The shoulder on peak 2 contains no chlorine, is not related to the drug, and is a mixture of endogenous metabolites).

Table 1 shows the concentration and relative amounts of CABA and N-acetyl-CABA in urine collected 45–60 min after the epidural administration of chloroprocaine. In the urine collected 2 hours later, the amount of N-acetyl-CABA had increased by four- five-fold, whereas the amount of CABA remained unchanged. This shows that N-acetyl-CABA gains dominance in the urine with time.

Figure 4 shows a gas chromatogram of methylated organic acids from urine of a newborn voided shortly after delivery. The dominating peak 2 is N-acetyl-CABA, identified by GC–MS. There was only small amounts of unmetabolized CABA (peak 1) present in the urine.

Discussion

The metabolism of para-aminobenzoic acid may follow a number of different pathways of which the dominating ones are believed to be acetylation and conjugation with glycine.[2] Although it has been stated that CABA is eliminated in the urine as a conjugate, its structure has not been previously identified. It has been suggested that the unknown metabolite may be a glycine conjugate, based on negative tests for sulfate and glucuronic acid conjugates.‡

The present study demonstrates, however, that the major metabolic route for the elimination of CABA is N-acetylation and subsequent excretion in the urine, and that glycine conjugation is not taking place at an appreciable rate. The large quantity of N-acetyl-CABA in the urine of a newborn, reflects either fetal acetylation of CABA or placental transfer of this metabolite from the mother. However, regardless of the origin of N-acetyl-CABA, the neonate seems quite capable of eliminating the metabolite, and this gives additional support to the use of chloroprocaine in obstetric analgesia and anesthesia.

‡ O'Brien JE, Abbey V, Hindsvark O, *et al*: personal communication.

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