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Short Communication

Determination of local anaesthetics in body fluids by gas chromatography with surface ionization detection

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ABSTRACT

Ten local anaesthetics were tested for their detection by gas chromatography (GC)–surface ionization detection (SID). Lidocaine, mepivacaine and bupivacaine were detected with the highest sensitivity; their detection limit was 5–10 pg in an injected volume. The sensitivity of other drugs, such as procaine, dibucaine, tetracaine and oxybuprocaine, was an order of magnitude lower than that of the above three local anaesthetics. A detailed procedure for isolation of local anesthaetics from human whole blood and cerebrospinal fluid (CSF) by the use of Sep-Pak C_{18} cartridges, before the GC–SID, is also presented. The recovery of lidocaine, mepivacaine and bupivacaine, which had been added to 1 ml of whole blood or CSF, was close to 100%.

INTRODUCTION

Surface ionization detection (SID) for gas chromatography (GC) was developed by Fujii and Arimoto in 1985 [1]. The new detection system was reported to be very specific and sensitive, especially for organic compounds with tertiary amino groups in their structures [1]. Application of GC–SID to drug analyses has recently begun and only a few studies have been reported on fentanyl [2], aprindine [3] and tricyclic antidepressants [4].

This paper describes the use of GC–SID to analyse ten local anaesthetics: three were found to be highly sensitive.

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EXPERIMENTAL

Materials

Lidocaine, procaine · HCl, dibucaine · HCl, ethyl *p*-aminobenzoate (benzocaine) and tetracaine were obtained from Sigma (St. Louis, MO, U.S.A.), mepivacaine · HCl, bupivacaine · HCl and propitocaine · HCl from Fujisawa Pharmaceutical (Osaka, Japan), oxybuprocaine · HCl from Santen Pharmaceutical (Osaka, Japan) and *p*-(butylamino)benzoic acid-2-(diethylamino)ethyl ester · HCl from Teikoku Chemical Industries (Osaka, Japan). Sep-Pak C_{18} cartridges were purchased from Waters (Milford, MA, U.S.A.). Other common chemicals used were of the highest purity commercially available. Whole blood was obtained from healthy subjects and cerebrospinal fluid (CSF) from cadavers at forensic autopsy.

Isolation with Sep-Pak C_{18} cartridges

Drugs were extracted on Sep-Pak C_{18} cartridges according to Suzuki *et al.* [5]. For pretreatment of a cartridge, 10 ml of chloroform–methanol (9:1), 10 ml of acetonitrile and 10 ml of distilled water were passed through it.

A 1-ml volume of whole blood or CSF, with or without addition of drugs, was mixed with 9 ml of distilled water and loaded on a Sep-Pak cartridge at a flow-rate not greater than 5 ml/min. The cartridge was washed with 10 ml of water, and finally 4 ml of chloroform–methanol (9:1) were passed through it to elute the local anaesthetics, which were collected in a vial. The eluate consisted of a major amount of an organic layer (lower phase) and a minor amount of an aqueous layer (upper phase); the latter was discarded by aspiration with a Pasteur pipette. The organic layer was evaporated to dryness under a stream of nitrogen, and the residue was dissolved in 100 μl of methanol. A 1-μl aliquot was subjected to GC analysis.

GC conditions

A Shimadzu GC-15A instrument equipped with an SID system with a non-polar fused-silica Ulbon HR-1 capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm, Chromato Packings Center, Shinwa-kako, Kyoto, Japan) and a split–splitless injector was used. The GC conditions were: column temperature, 100–280°C (10°C/min); injection temperature, 200°C; helium flow-rate, 22.5 cm/s. The SID conditions were: heating current through the platinum emitter, 2.2 A; emitter temperature, *ca.* 600°C; ring electrode bias voltage, +200 V with respect to the collector electrode. The samples were injected in the splitless mode at a column temperature of 100°C, and splitter was opened after 2 min.

RESULTS AND DISCUSSION

The ten local anaesthetics tested were relatively heat-stable and did not decom-

pose during capillary GC analysis. The sensitivity to SID varied remarkably. The highest sensitivity was apparent with lidocaine, mepivacaine and bupivacaine; other drugs showed much lower sensitivity. Thus additional tests with whole blood and CSF were performed on these three compounds.

Fig. 1 shows gas chromatograms for whole blood and CSF extracts with and

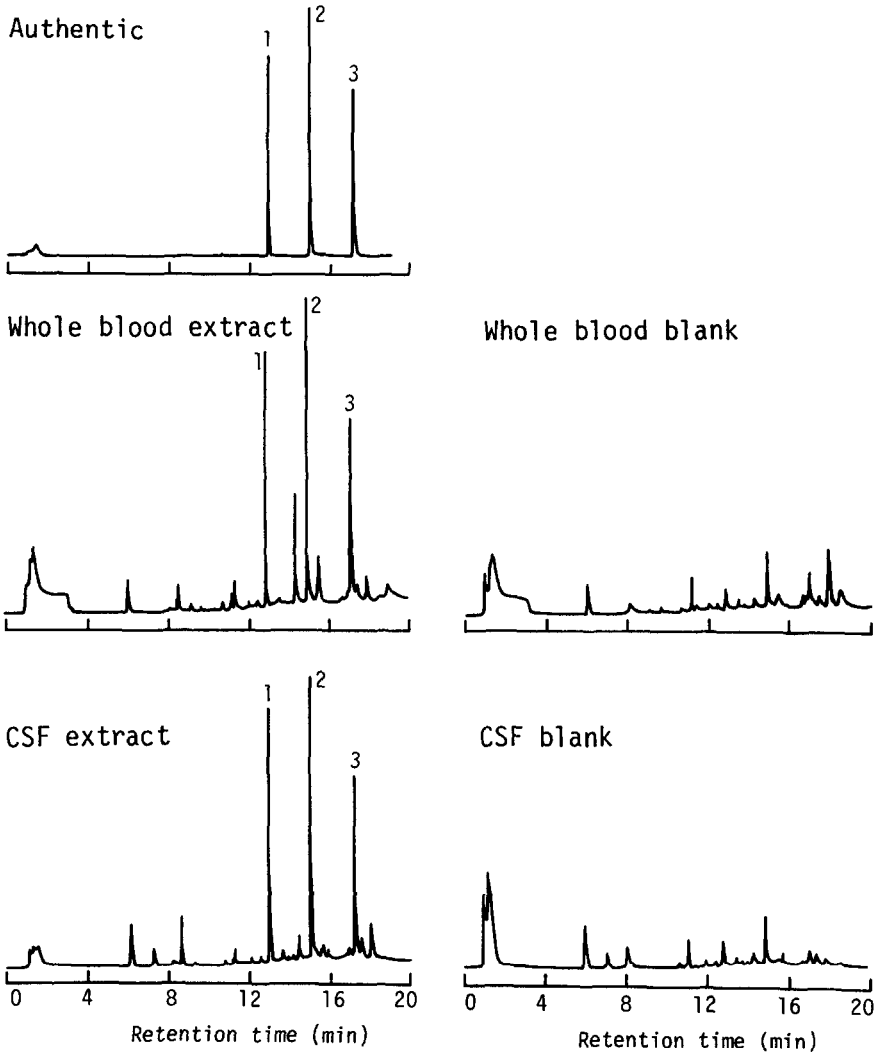

Fig. 1. Capillary GC–SID for human whole blood and CSF extracts in the presence and absence of local anaesthetics with use of Sep-Pak C_{18} cartridges. Peaks: 1 = lidocaine; 2 = mepivacaine; 3 = bupivacaine. GC was carried out with a Ulbon HR-1 fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm). The GC conditions were: column temperature, 100–280°C (10°C/min); injection temperature, 200°C; helium flow-rate, 22.5 cm/s. The samples were injected in the splitless mode at a column temperature of 100°C and splitter was opened after 2 min. The mixture of three local anaesthetics (5 ng each) was added to 1 ml of whole blood or CSF.

without addition of 5 ng each of the three compounds to 1-ml samples. Although there were some small impurity peaks overlapping those of the test compounds, their contamination gave almost no problems even at this low concentration. The recovery of the three anaesthetics was close to 100%.

Fig. 2 shows calibration curves for lidocaine, mepivacaine and bupivacaine. They showed excellent linearity in the range 10–100 pg in an injected volume. The equations and r values for the curves were: $y = 0.521x - 0.344$, $r = 0.9993$ for lidocaine; $y = 0.625x - 1.709$, $r = 0.9993$ for mepivacaine; and $y = 0.429x + 0.014$, $r = 0.9993$ for bupivacaine. The detection limit of each drug was 0.5–1.0 ng per ml of sample (5–10 pg in an injected volume). The sensitivity for procaine, dibucaine, tetracaine, oxybuprocaine and *p*-(butylamino)benzoic acid-2-(diethylamino)ethyl ester was *ca.* 10 times lower, and that for ethyl *p*-aminobenzoate and propitocaine was *ca.* 100 times lower.

The GC–SID sensitivity for lidocaine, mepivacaine and bupivacaine is an order of magnitude better than that obtained with GC with a nitrogen–phosphorus detector [6–10] or with an electron-capture detector [11], and three orders of magnitude higher than that obtained with GC with flame ionization detection (unpublished results). In our previous study, we also reported that SID was *ca.* 10 times more sensitive than thermionic ionization detection for some tricyclic antidepressants [4].

Fujii and Arimoto [1] explained that the sensitivity of SID is dependent on the ionization potential of the species as well as on the yield of the species generated through chemical reaction on the surface; organic compounds such as tertiary

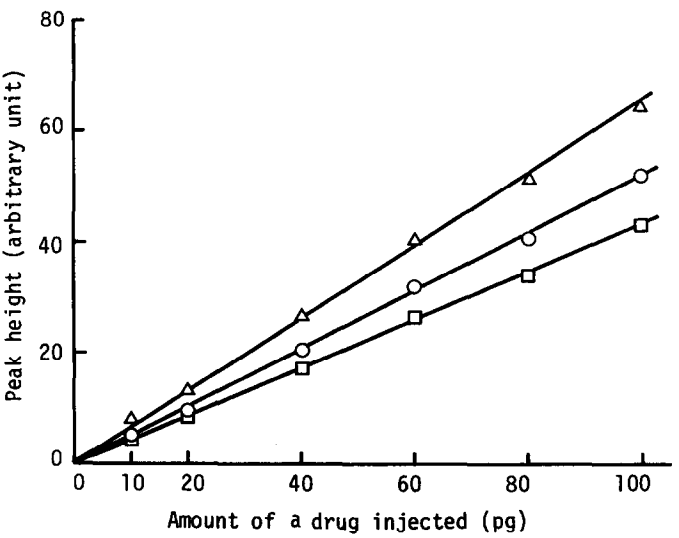

Fig. 2. GC–SID calibration curves for lidocaine (o), mepivacaine (△) and bupivacaine (□). GC conditions were as specified in Fig. 1.

amines, which form their dissociative species at low ionization potential, could be analysed by this method with extremely high sensitivity. An amide group, if present in a chemical structure, acts to lower its sensitivity [12]. However, in the present study, lidocaine, mepivacaine and bupivacaine showed extremely high sensitivity in spite of the presence of an amide group in each structure. It should be pointed out that all three anaesthetics contain a 2,6-dimethylphenylamide group together with a tertiary amino group. More studies should be done on various drug groups to define the molecular structures that result in high and low sensitivity to this new method.

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