A Simple Method for Gas Chromatographic Determination of Lidocaine in Tissues

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A simple gas chromatographic method for the determination of lidocaine in tissues is described. Lidocaine is extracted from the tissue samples using liquid nitrogen. The recoveries of lidocaine from brain, liver, and muscle were 98.6, 99.8, and 89.1 per cent, respectively. Results were reproducible to within 1.0 per cent of lidocaine administered. (Key words: Anesthetics, local, lidocaine; Measurement techniques, gas chromatography.)

LIDOCAINE has been investigated widely as a local anesthetic, cardiac antiarrhythmic agent, and an adjuvant for general anesthesia. The quantitative determination of lidocaine in tissue is obviously important for assessment of the distribution, metabolism, and toxicity of the drug following various routes of administration. Although several gas chromatographic assays of lidocaine have been reported, most of them are analyses of only whole blood or plasma.1–4 Benowitz and Roland7 described a rapid, sensitive method for measuring lidocaine in tissues, but it requires a large amount of tissue in one sample, thereby precluding serial measurement, especially in small animals. Keenanahan and Boyes8 measured unchanged lidocaine in rat tissue by a wet oxidative technique but interfering gas chromatographic peaks in carcass extracts made it impossible to determine the quantity of lidocaine. Ahmad and Medzihradsky9 reported a complex method for analysis of lidocaine in tissues. This method requires several steps, including deproteinization, ether wash, methylene chloride extraction, and evaporation. In this paper we report a method for extracting lidocaine from tissue samples using liquid nitrogen and determining its concentration by a modification of the method of Ishikawa.4 Our method is simpler, more rapid, and enables quantification of lidocaine concentrations as low as 0.5 μg/g in approximately 0.3-g samples of tissue.

Methods

The gas chromatographic unit used was a Shimazu GC-3BF® with a hydrogen-flame ionization detector. The chromatographic column, a coiled glass tube 300 cm long and 3.0 mm ID, was packed with Infusorial Earth 80/100 mesh coated with OV-17, 3 per cent by weight. The flow rates of nitrogen and hydrogen gases were 68 and 45 ml/min, respectively. Column and injection temperatures were 235 and 285 C, respectively. The internal standard used was mepivacaine, 25–50 μg.

Samples of fresh tissue (brain, liver, muscle) of approximately 0.3 g each were obtained from mongrel dogs undergoing lidocaine infusion. The samples were frozen immediately in liquid nitrogen. After crushing the frozen tissue with a precooled piston, the powdered samples were weighed accurately in a 10-ml preweighed homogenizer tube. A 3-ml volume of perchloric acid, 0.6 N, and mepivacaine were added. The amount of mepivacaine was adjusted in accordance with the expected tissue lidocaine concentration. After homogenization and centrifugation at 4,500 rpm for 10 minutes, the supernatant was transferred to a glass centrifuge tube. A 2-ml volume of perchloric acid, 0.2 N, was added to the residue and homogenization and centrifugation were repeated. The combined supernatants were adjusted to pH 6.5 with potassium hydroxide, 5 N. Then crystallized potassium perchlorate was removed by centrifugation.

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Fig. 1. Specimen record of a gas chromatogram, showing peaks of lidocaine (80.0 μg/g) and mepivacaine (50.0 μg/g). Tissue sample was 0.5 g of canine brain.
ugation. A 2-ml volume of solution was alkaliized close to pH 13 with sodium hydroxide, 2 N, and added to 2 ml chloroform. The mixture was centrifuged at 2,500 rpm for 10 minutes. The aqueous alkaline layer was aspirated off and discarded. The chloroform layer was condensed to about one-tenth volume and one to a 1–3-μl volume of this layer was injected into the gas chromatograph with a 10-μl microsyringe. The peak-height ratio of lidocaine to mepivacaine was measured and the concentration of lidocaine was determined with reference to a standard calibration curve.

**Results**

A specimen record of a gas chromatogram of a brain tissue sample is shown in figure 1. The retention time for lidocaine was 6 minutes and that for mepivacaine, 12 minutes. No interference with the assay was detected. Repeated injection could be performed at 15-minute intervals. The standard calibration curves are shown in figure 2. When the weight ratio was less than 0.2, calibration curve 1 was obtained, and when the ratio was more than 0.2, calibration curve 2 was obtained. In both, good correlation between the weight ratio and the peak height ratio was obtained. The determination of lidocaine was made in concentrations ranging from 0.5 to 200 μg/g tissue. Recoveries of lidocaine from brain, liver, and muscle were 98.6, 99.8, and 89.1 per cent, respectively. The results were reproducible to within 1.0 per cent of administered lidocaine. Thus, this method is simple and reliable, and can be used for repeated determinations of lidocaine concentrations in various tissues.

**References**