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DETERMINATION OF LIDOCAINE AND ACTIVE METABOLITES IN BLOOD SERUM BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

Oxidation of lidocaine and its principal metabolites, monoethylglycine xylidide and glycine xylidide, at glassy carbon electrodes was employed to permit electrochemical detection of these compounds following separation by high-performance liquid chromatography. The absolute detection limits found for these compounds were 2 ng, 5 ng, and 4 ng injected, respectively. The resulting assay was suitable for the routine quantitation of lidocaine and these metabolites in blood serum over the entire therapeutic range, 1—6 μg/ml. Total analysis time is 10—15 min.

INTRODUCTION

Lidocaine (Fig. 1) belongs to a group of aromatic amides widely used as local anesthetics. Lidocaine also possesses antiarrhythmic properties and is frequently used as a therapeutic agent in the treatment of cardiac disorders. For lidocaine to be effective in this latter capacity, it is necessary to achieve blood serum levels of 1—6 μg/ml [1, 2]. Below this range, the drug is ineffective, whereas serum levels greater than 6 μg/ml produce toxic effects. Two of its metabolites, monoethylglycine xylidide (MEGX) and glycine xylidide (GX) (Fig. 1), also possess antiarrhythmic activity and/or central nervous system

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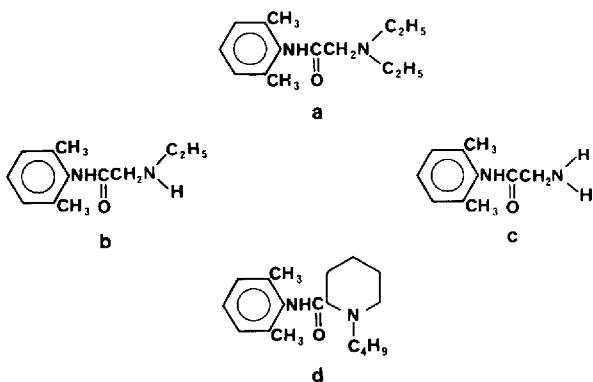


Fig. 1. Structure of (a) lidocaine, (b) MEGX, (c) GX, and (d) bupivacaine.

toxicity [3—6]. Because significant concentrations of these metabolites may be achieved in patients receiving lidocaine treatment, it is desirable to monitor serum levels of all three compounds conveniently and accurately.

Numerous gas—liquid chromatographic approaches have been described for this purpose [7—14]. These methods, employing flame ionization, mass spectroscopic, or nitrogen—phosphorus detection, have been shown to possess excellent sensitivity for lidocaine, generally in the 1—10 ng/ml range. But, in many procedures, the metabolites MEGX and GX either are not determined at all or are not separately distinguished from lidocaine. More importantly, the application of all of these approaches to the routine analysis of real serum samples is limited by the sample preparation time taken up by the lengthy derivatization, extraction, or evaporation/preconcentration procedures required. Recently, several high-performance liquid chromatography (HPLC) methods using ultraviolet (UV) detection have been employed [15—18]. Unfortunately, as these compounds contain no chromophore which absorbs strongly in the visible or near-UV regions, the wavelength employed for detection must be approximately 200 nm. The most sensitive of the HPLC methods reported to date has a detection limit of 20 ng/ml (or 2 ng injected) for lidocaine, an order of magnitude poorer than the most sensitive of the gas chromatographic methods [16]. Sample preparation for HPLC is generally less lengthy and involved than that case for gas chromatography; however, a minimum of two extraction steps is still required even for the simplest HPLC procedure.

In recent years, amperometric electrochemical detection following liquid chromatography (LC—ED) has become increasingly popular for the quantitation of easily oxidizable analytes. Its principal advantages include uniformly high sensitivity and a unique selectivity toward compounds that can be electrolyzed at the applied detector potential [19]. To our knowledge, the electrochemical behavior of lidocaine, MEGX, and GX has not yet been reported. But, since these compounds all possess highly substituted aromatic centers, they might be expected to undergo oxidation readily. In this work, we will describe the anodic electrochemistry of these compounds at graphite electrodes and the development of an LC—ED procedure for their determination. Sufficient selectivity and sensitivity have been attained to permit the

procedure to be useful for routine clinical monitoring of lidocaine and its metabolites in serum with minimal sample preparation.

EXPERIMENTAL

Reagents

Lidocaine, MEGX, and GX were obtained from Astra Pharmaceutical; bupivacaine (Marcaine®), used as an internal standard, was obtained from Sterling-Winthrop Research Institute, a division of Sterling Drug. All compounds were provided as HCl salts. HPLC grade water and acetonitrile were purchased from Burdick & Jackson Labs. Buffer salts, ammonium hydroxide, and phosphoric acid were purchased from Fisher Scientific and were used as received without further purification.

Voltammetry

Cyclic voltammetry was performed with a Bioanalytical Systems (West Lafayette, IN, U.S.A.) Model CV-1B potentiostat. Either a glassy carbon or a carbon paste working electrode, a saturated Ag/AgCl reference electrode, and a platinum wire counter electrode were used for all experiments. The supporting electrolyte consisted of 40 m*M* ammonium phosphate adjusted to the desired pH or a binary mixture of this buffer and acetonitrile. Experiments were performed at a scan rate of 30 mV/sec.

Chromatography

The HPLC system consisted of a DuPont (Wilmington, DE, U.S.A.) Model 8800 pump, a Rheodyne (Berkeley, CA, U.S.A.) Model 7125 sample injector with a 50-μl sample loop, a Bioanalytical Systems LC-4B amperometric detector equipped with a Model TL-5 thin-layer glassy carbon working electrode and an Ag/AgCl reference electrode, and a Spectra-Physics (Arlington Heights, IL, U.S.A.) Model SP4100 computing integrator.

Chromatographic experiments were performed on a 30-cm, 10-μm silica column (Regis, Morton Grove, IL, U.S.A.). The mobile phase consisted of acetonitrile—40 m*M* ammonium phosphate (pH 7.8) (22—25:75—78). A 3-cm silica cartridge (Brownlee Labs., Santa Clara, CA, U.S.A.) was placed ahead of the analytical column as a guard. The flow-rate used for all experiments was 2.0 ml/min.

Sample preparation

A fresh octadecylsilane Sep-Pak cartridge (Waters Associates, Milford, MA, U.S.A.) was initially prepared by flushing with 6 ml of acetonitrile and then 6 ml of water. A 100-μl volume of bupivacaine internal standard was added to 1 ml serum, followed by 400 μl of water (or lidocaine and metabolites for spiked samples). The tube containing these constituents was vortex-mixed for 30 sec. Three drops of 0.15 *M* ammonium hydroxide were then added to convert the compounds to their unprotonated forms. After the solution was again vortex-mixed, the entire sample was passed through the Sep-Pak cartridge. The Sep-Pak was flushed with 3 ml of water, and the components of interest were eluted with 2 ml of acetonitrile. The eluent was then injected into

the HPLC system through a Swinney filter containing a 13-mm diameter, 0.45-μm Nylon 66 filter.

RESULTS AND DISCUSSION

Electrochemistry

Numerous previous investigations [20] have focused on the anodic electrode reactions of aromatic amines and many derivatives substituted variously at both nitrogen and ring positions. These compounds generally exhibit anodic waves within the potential range between +0.5 and +1.0 V vs. Ag/AgCl. These waves, which presumably involve an initial one-electron oxidation to produce a cation radical with the charge residing primarily on the amine nitrogen, are nearly always irreversible because of rapid follow-up coupling reactions that produce dimeric and polymeric products. To our knowledge, no information has yet been reported for aromatic amides directly related to lidocaine or the other compounds of interest here. However, some general similarities to aromatic amines were expected for these compounds.

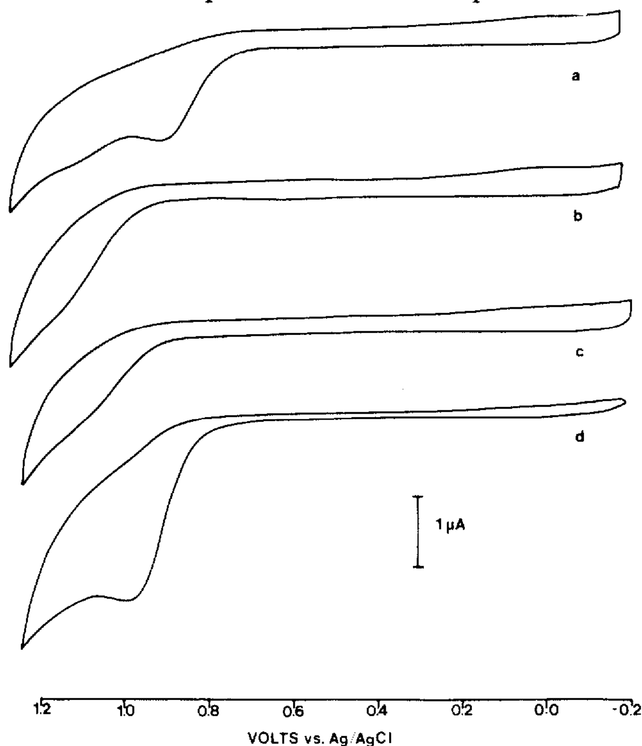


Fig. 2. Cyclic voltammograms of (a) $1.0 \cdot 10^{-4}$ *M* lidocaine, (b) $1.0 \cdot 10^{-4}$ *M* MEGX, (c) $1.0 \cdot 10^{-4}$ *M* GX, and (d) $1.0 \cdot 10^{-4}$ *M* bupivacaine at a glassy carbon electrode. Electrolyte—buffer: 10% acetonitrile—90% ammonium phosphate (pH 7.4).

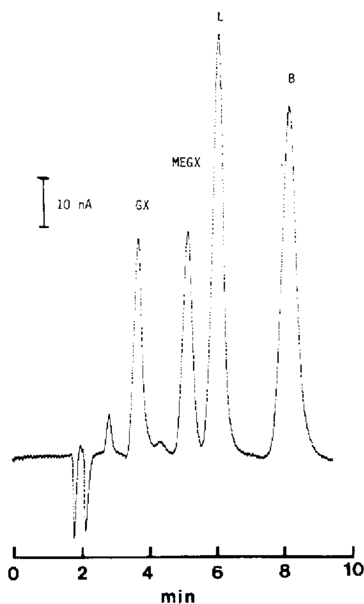
Fig. 2 shows the cyclic voltammograms obtained for lidocaine, MEGX, GX, and bupivacaine, which was used as an internal standard in this assay. (The structure of bupivacaine is also shown in Fig. 1.) All exhibited irreversible

oxidation waves; at pH 7.4 the peak potentials for lidocaine and bupivacaine occurred betweeen +0.90 and +1.00 V vs. Ag/AgCl while those for GX and MEGX occurred only at significantly higher potentials in the vicinity of +1.10 V. In fact, at the analyte concentrations employed, the anodic waves for the latter compounds were only barely observed before the onset of solvent oxidation. In all cases, no reduction waves were evident on the reverse cathodic scan. Subsequent cycles over the same potential range showed no drastically different behavior. All of the oxidation waves were found to shift to higher potentials as the pH was decreased, with the shift for lidocaine amounting to approximately 75 mV per pH unit up to its pK_a, 7.7. Similarly, the waves again shifted anodically and also became noticeably broader when increasing proportions of acetonitrile were added to the electrolysis medium. When the cyclic voltammetric behavior of this series of compounds was measured at a carbon paste electrode, no differences in behavior were noted.

Chromatography

On the basis of the electrochemical information described above for lidocaine and its metabolites, these compounds clearly do not represent ideal candidates for LC—ED. The potential required for their oxidation is considerably higher than that desirable to achieve both ultimate sensitivity and reasonable selectivity. In fact, detection of GX and MEGX by LC—ED could be expected to necessitate detector potentials of the order of +1.20 V vs. Ag/AgCl. However, for routine clinical applications which require the assay of these compounds only at the μg/ml level, high sensitivity comparable to that ordinarily expected of LC—ED is really not essential.

The cyclic voltammetry results indicate that optimum electrochemical conditions in LC—ED would be favored by an alkaline mobile phase of relatively low organic content. Both of these factors will serve to decrease the oxidation potential of the analytes to as low a value as is practical. Unfortunately, in conventional reversed-phase chromatography employing an octadecylsilane column, high pH conditions required that a relatively high acetonitrile (or other organic solvent) content be employed in the mobile phase in order to elute lidocaine and the other compounds within a reasonably short retention time. Preliminary work using this approach (e.g. acetonitrile—pH 7.4 buffer, 1:1) produced chromatograms in which the lidocaine response consisted of a broad, severely tailing peak and the MEGX and GX gave no observable signal at all. An alternate approach, described recently by Bidlingmeyer et al. [21] for the separation of organic amines including lidocaine, consists of the use of a bare silica gel stationary phase in conjunction with an aqueous—acetonitrile eluent. This somewhat unconventional reversed-phase procedure, for which the retention mechanism is thought to consist of a direct interaction of silanol groups on the column material with the amine function of the analyte, was shown not only to produce narrow, symmetrical peaks but also to be compatible with elution by the high pH, low acetonitrile mobile phase required for optimum LC—ED detection. Initial trials employing this latter chromatographic approach immediately produced favorable results for lidocaine, MEGX, and GX. Consequently, the approach was employed in all subsequent work reported here.



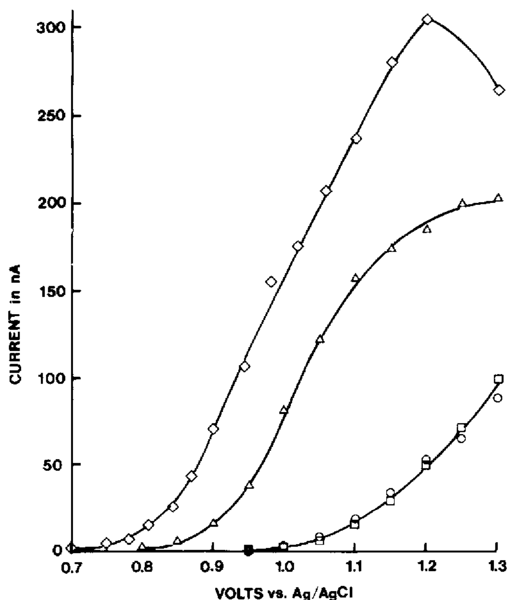


Fig. 3. Chromatogram of standard mixture of lidocaine (L) and metabolites (B = bupivacaine). All concentrations are $1.0 \cdot 10^{-5}$ *M*. Mobile phase: acetonitrile—ammonium phosphate buffer (pH 7.8) (22:78); *E* = +1.20 V vs. Ag/AgCl.

Fig. 4. Hydrodyanmic voltammogram of lidocaine (△), MEGX (□), GX (○), and bupivacaine (◇). All concentrations are $1.0 \cdot 10^{-4}$ *M*; other conditions as for Fig. 3.

The chromatogram shown in Fig. 3 was obtained for a standard solution of lidocaine, MEGX, GX, and bupivacaine at a detector potential of +1.20 V vs. Ag/AgCl. Under the conditions employed, the peaks corresponding to all four compounds were completely resolved. The response of the system as a function of detector potential was demonstrated by obtaining hydrodynamic voltammograms (i.e. profiles of chromatographic peak current vs. applied potential) for each of the analytes. These voltammograms are shown in Fig. 4. It can be seen that a potential of +1.20 V is required if GX and MEGX are to be detected. However, if only lidocaine were of interest, a potential of some 0.2 V less would be equally suitable. The hydrodynamic voltammograms match closely the results anticipated on the basis of the cyclic voltammograms reported above. Detection limits (i.e. signal-to-noise ratio = 2) for standard solutions were approximately 2 ng injected for lidocaine, 5 ng for MEGX, and 4 ng for GX.

Blood serum analysis

The chromatogram of a blood serum blank obtained from a patient prior to intravenous lidocaine infusion and subjected to the Sep-Pak treatment process described in the Experimental section is shown in Fig. 5A. The intense peaks eluting between 1 and 3 min corresponded to serum components weakly retained under the chromatographic conditions employed and occurred consistently for all serum samples examined. The remaining peak at a retention time of 4.5 min was found in varying intensity for all samples — serum,

standards, or even deionized water — eluted through the octadecylsilane Sep-Paks. Numerous Sep-Paks were examined, and various procedures for pretreating the Sep-Paks prior to the serum assay were investigated. Although extensive preliminary flushing of the Sep-Pak generally served to reduce the intensity of the interfering peak considerably, no procedure was successful in removing it consistently and completely. Gas chromatography—mass spectrometry performed on eluent fractions collected from the Sep-Pak indicated the presence of several compounds including bis(2-ethylhexyl)-phthalate and 2,6-di-*tert.*-butyl-4-methylphenol. However, the identity of the compound causing the peak was not determined definitely. Since the unidentified substance did not coelute with any of the compounds of interest, its effect on the lidocaine assay was not significant. No further measures were taken to eliminate it completely.

Calibration curves were prepared from quadruplicate serum samples spiked with lidocaine, MEGX, and GX from 0.20 to 12 μg/ml and subjected to the Sep-Pak treatment. The signal taken for each consisted of the ratio of the peak height obtained for the drug or metabolite to that of the bupivacaine internal standard. In all cases, the calibration curves were linear down to the detection limit of the individual compound. Least-squares analysis of the calibration curves yielded the following characteristics: for lidocaine, slope = 2.21, y-inter-

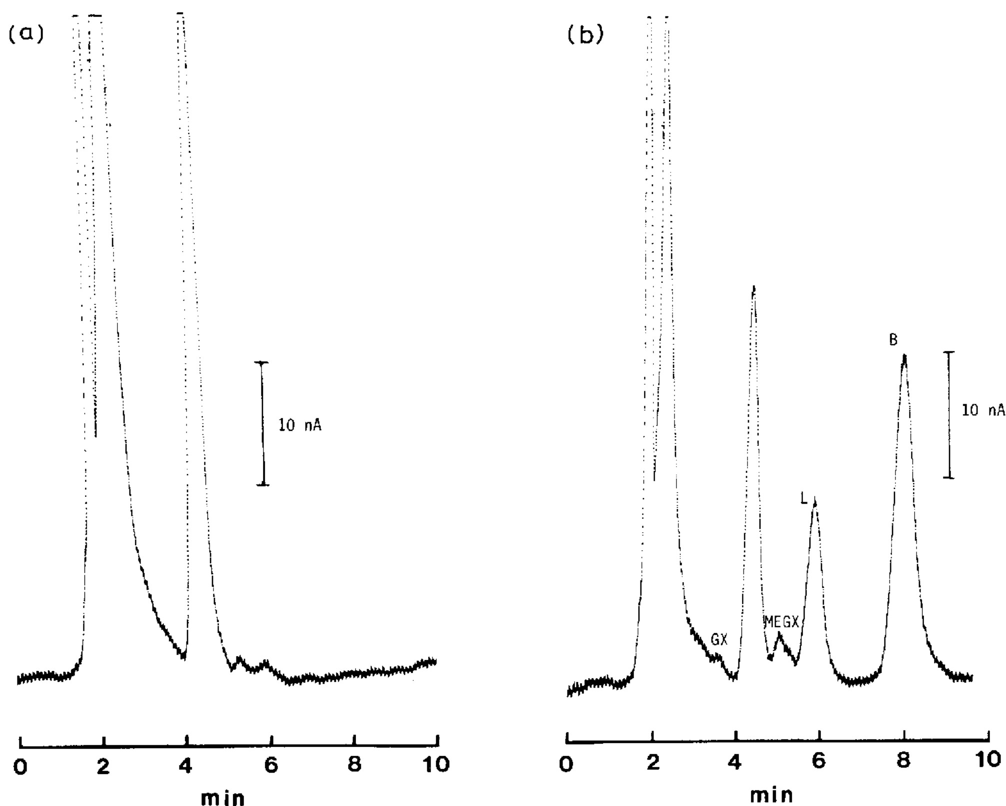


Fig. 5. Chromatograms of blood serum sample obtained (a) prior to lidocaine treatment and (b) 20 min after lidocaine injection. Conditions as for Fig. 3.

TABLE I

DETERMINATION OF LIDOCAINE IN BLOOD SERUM OF PATIENTS FOLLOWING 200-mg INJECTION

Patient	Lidocaine concentration (μg/ml)			
	5 min post injection	10 min post injection	1 h post injection	2 h post injections
A	2.62	0.82	0.64	0.55
B	4.53	2.55	0.89	0.78
C	3.82	2.20	1.45	1.14
D	4.65	2.21	1.50	0.96
E	6.66	3.27	2.62	2.48
F	3.50	0.66	0.48	—
G	2.55	0.95	0.49	0.24

cept = −0.116, correlation coefficient = 0.997; for MEGX, slope = 1.04, y-intercept = 0.056, correlation coefficient = 0.996; and for GX, slope = 0.718, y-intercept = −0.054, correlation coefficient = 0.991. Reproducibility, measured in terms of the relative standard deviation for four trials, was generally 5—10%. Recoveries of the drugs and metabolites and respective detection limits in serum were as follows: for lidocaine, recovery = 76% and detection limit = 0.2 μg/ml of blood serum; for MEGX, 60% and 0.6 μg/ml; for GX, 44% and 0.5 μg/ml. The typical analysis time required for quantitation of all three species was 10 min.

Serum samples were obtained from several patients at intervals of 5 min, 20 min, 1 h, and 2 h following a 200-mg lidocaine intravenous injection. The pharmacokinetic data for seven patients are summarized in Table I, and Fig. 5B shows a typical chromatogram corresponding to a 20-min post-injection serum sample where peaks for MEGX and GX are just beginning to appear. The indicated serum concentrations were all well within the range expected following a 200-mg injection but show the wide inter-patient variability which can be a concern even with identical dosages.

CONCLUSIONS

The method described is sufficiently rapid, selective, and sensitive to be appropriate for quantitating lidocaine, MEGX, and GX levels in patients treated for cardiac arrhythmias. Samples may be prepared quickly and simply enough to permit convenient real-time monitoring in patients undergoing lidocaine treatment.

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