The Effect of Preanodization on the Electrochemical Detection of Oxprenolol in Plasma

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Key Words
Liquid chromatography
Electrochemical detection
Oxprenolol
Pre-anodization
Increased sensitivity

Summary
Modification of the electrode surface by pre-anodization of glassy carbon electrodes causes a significant increase in sensitivity for the detection of oxprenolol. This method was used in conjunction with HPLC to measure the concentration of oxprenolol in samples of plasma and the results compared with those obtained by the established electron-capture GC method. The resulting values showed a good correlation between the methods and indicate that pre-anodization is a practical means for the enhancement of electrochemical detector sensitivity.

Introduction
Electrochemical detection combined with high-performance liquid chromatography (LCEC) has been shown to offer a useful approach to the detection of compounds by oxidation or reduction [1, 2]. A limitation of LCEC arises from the electrode potential required to oxidize or reduce the compound of interest. For instance, much LCEC work has been carried out with compounds containing easily oxidized species such as phenolic groups, phenothiazine sulphur atoms, imidazole nitrogen and secondary and tertiary aliphatic amines, all of which can be oxidized at applied potentials of less than +1.2V. However if oxidation of a compound requires applied potentials greater than +1.2V there is a problem of high background current due to the oxidation of the aqueous component of the mobile phase. Some encouraging work has been carried out with LCEC in non-aqueous mobile phases and normal phase HPLC [3] but not every compound is capable of analysis by this approach.

The problem of high applied potentials has been partially overcome by the use of electrochemical pretreatment of glassy carbon working electrodes. Such a technique has enabled the oxidation of hydrazine at applied potentials of +1.0V instead of the more usual +1.5V [4]. A different preanodization technique has also used to enable the detection of oxprenolol at +1.20V instead of +1.32V [5] and it is the practical application of the latter method which forms the basis of this study.

Materials and Methods
Reagents and Materials
Oxprenolol hydrochloride was supplied by Ciba-Geigy (Horsham, UK), timolol maleate by Merck, Sharp and Dohme (UK) and metoprolol tartrate from Geigy Pharmaceuticals (Macclesfield, UK). Stock solutions of 100/~g/ml were prepared in distilled water and could be stored for more than a month at +4°C. More dilute solutions were prepared daily from the stock solutions. HPLC grade methanol (Fisons, Loughborough, UK) was used in the mobile phase; diethyl ether (May and Baker p.o. quality), SLR grade hexane and analytical grade toluene (Fisons, Loughborough, UK) were distilled before use, the ether and hexane then being equilibrated with distilled water. Trifluoroacetic anhydride was supplied by Sigma Ltd. (Poole, Dorset, UK). All other reagents were prepared from analytical grade chemicals.

Subjects
Four healthy young volunteers were recruited and all were shown to have normal haematological and biochemical profiles immediately before starting the study. The nature and purpose of the investigation was explained to each, and written consent obtained. The protocol described to them had been approved by the Central Birmingham Health District Research Committee. Each volunteer attended in a fasting state and took orally 160mg of oxprenolol hydrochloride in the form of 2 x 80mg Trasicor (R) tablets. Blood was withdrawn via a cannula, inserted into a forearm vein at the following times: 0 (immediately before tablet administration) 0.5, 1, 1.5, 2, 3, 4, 6, 12 and 24 hours.
Plasma was obtained by centrifugation and stored at −20°C until analysed by the methods described.

Chromatography

Gas-liquid chromatography (GLC) was based on a published method for the assay of oxprenolol [6], using a Hewlett-Packard series 5700A chromatograph fitted with a 63Ni electron-capture detector. A glass column, 2m long x 3mm i.d. was packed with 3% OV1 on Gas-Chrom Q 100–120 mesh (Perkin-Elmer, Beaconsfield, Bucks, UK). Oven temperature was 190°C, injection port 250°C, detector 300°C. The flow rate for the carrier gas (argon-methane 9:1) was maintained at 40ml/min. The internal standard was metoprolol tartrate.

High-performance liquid chromatography was performed using a Waters Model 6000A solvent delivery system, attached to a stainless-steel column, 25cm x 4.6mm i.d. packed with 5μm Whatman PXS 5/25 Partisil ODS 3, (Whatman Chemical Separations, Maidstone, UK). Samples were introduced onto the column by means of a 100μl Rheodyne loop injector. The mobile phase was a mixture of methanol:0.2M sodium dihydrogen phosphate:88% orthophosphoric acid (Sp.Gr. 1.75):water (450:200:3:347) pumped at a flow-rate of 1.0ml/min. Detection was carried out electrochemically, using a TL5A thin-layer assembly with an LC2A controller (Bioanalytical Systems, Indiana, USA) working at an applied potential of +1.20V versus a silver/silver chloride reference electrode.

Preanodization of the Working Electrode

A mixture of 40mM sodium dihydrogen phosphate in 0.3% (v/v) orthophosphoric acid was slowly introduced into the thin-layer assembly taking care to avoid air bubbles. The working electrode was connected to the positive terminal and the auxiliary electrode to the negative terminal of a d.c. power supply (Thurlby Electronics Ltd., Huntingdon, UK). The current was applied for 2 minutes at 6.0V and a maximum of 2mA. Normally a current of less than 1mA was recorded, any current greater than 2mA caused damage to the working electrode when this particular solution was present in the thin-layer assembly. After two minutes the TL5A system was reconnected to the control unit and HPLC pump, and the pumping of the mobile phase was begun. The detector was allowed to equilibrate at an applied potential of +1.32V and was capable of providing a stable and enhanced response to 100ng samples of timolol and oxprenolol for several hours. Similarly stable responses could be obtained at applied potentials of +1.20V, but only if the electrodes had been equilibrated at +1.32V beforehand. The injection of larger samples of oxprenolol gave rise to some loss of sensitivity which could be avoided by injecting 5μg samples of timolol and oxprenolol whilst the detector was set at an applied potential of +1.32V.

Extraction of Oxprenolol From Plasma for HPLC Assay

Sonicated plasma (0.5ml) was added to glass centrifuge tubes, followed by 0.1ml of timolol maleate (internal standard, 1.0μg/ml) followed by 0.5ml of 0.2M sodium hydroxide solution, 0.8g of solid sodium chloride and 4ml of water-equilibrated diethyl ether. The mixture was shaken gently for 15 minutes, centrifuged at 300g for 10 minutes, and the organic phase removed to a conical glass centrifuge tube. A further 4ml of diethyl ether were added to the plasma mixture and the procedure repeated. The organic phases were pooled, 0.25ml of 0.1% (v/v) orthophosphoric acid was added, followed by vortexing and centrifugation at 300g for 10 minutes. The organic phase was discarded and the acid was washed with 2ml of water-equilibrated hexane, vortexed and centrifuged as described above. The hexane was discarded and 100μl samples of the orthophosphoric acid phase were injected onto the HPLC/Preanodized detector system under the conditions previously described.

Results and Discussion

The advantages of preanodization are illustrated in Table I where it can be seen that the detector is as sensitive at +1.20V when preanodized as it is at +1.32V under normal conditions. Since it is often difficult to work with applied voltages greater than +1.20V the usefulness of preanodization is obvious.

Typical HPLC chromatograms of extracts from volunteer plasma at zero time (A) and after the administration of oxprenolol (B) are shown in Fig. 1. Under the conditions described in the text the retention times for timolol and timolol maleate were 1.25 and 2.00 minutes, respectively.

<table>
<thead>
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<th>Table I. The effect of preanodization of electrochemical sensitivity.</th>
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<td>Applied Potential (V)</td>
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Values obtained from repeated injection of 100ng each of oxprenolol and timolol.

Fig. 1

HPLC/LCEC Traces from blank plasma (A) and plasma taken 3 hours after the administration of 160mg of oxprenolol. Peak 1 was timolol internal standard, peak 2 was oxprenolol. Experimental details shown in the text.