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Electrochemical behaviour of tizanidine at solid electrodes*

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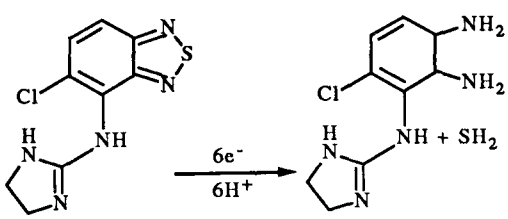
Abstract: The electrochemical behaviour of tizanidine [5-chloro(Δ-2-imidazolinyl-2-amino)-4-benzothiadiazole-2,1,3], a centrally-active skeletal muscle relaxant has been investigated in aqueous media at the carbon paste electrode (CPE). Cyclic voltammetry at different pH values, controlled potential coulometry and comparative studies on three structurally related molecules have permitted identification of the oxidation site of tizanidine and suggest possible oxidation products in acidic media. The electrochemical reduction at the CPE occurred in one irreversible step and the reduction product (diamine derivative) was detected and characterized on the positive going scan in cyclic voltammetry. Quantitative measurements of tizanidine within the range 2×10^{-5} M and 1×10^{-4} M have been realized at the CPE using the differential pulse technique.

Keywords: *Tizanidine; carbon paste electrode; electrochemistry.*

Introduction

Tizanidine [5-chloro(Δ-2-imidazolinyl-2-amino)-4-benzothiadiazole-2,1,3] (Fig. 1, structure I) is a centrally-active skeletal muscle relaxant intended for the symptomatic treatment of acute muscle spasms and of chronic spasticity [1–4]. Literature data on metabolic studies in man, rat, dog, rabbit and mouse showed extensive biotransformation of tizanidine mainly by oxidative routes [5]. It was thus of interest, for comparative purposes, to investigate the electrochemical behaviour of tizanidine. The molecule has been previously studied by dc, ac and pulse polarography and the electrode process has been characterized [6]. The electrochemical mechanism involved a six electron transfer with opening of the thiadiazole moiety and liberation of H_2S [6] [equation (1)]. The present work has been realized on solid electrodes in order to explore both oxidation and reduction mechanisms. Structurally related molecules were investigated under identical experimental conditions in



Equation 1

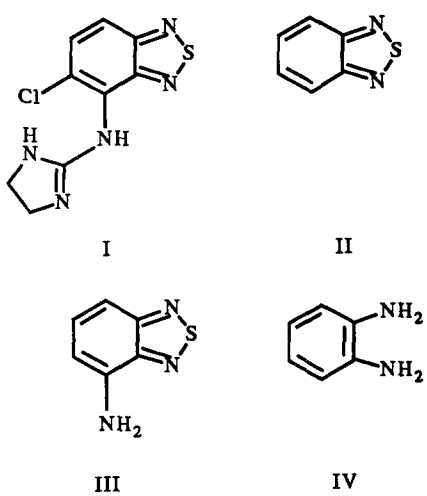


Figure 1
Structure of tizanidine and of some analogues.

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✠This paper is dedicated to the memory of Professor G.J. Patriarche.

order to try to understand the redox behaviour of tizanidine at the carbon paste electrode (CPE).

Experimental

Apparatus

Voltammetric measurements were made with a Model 175 Universal programmer/ Model 174 potentiostat (Princeton Applied Research) and a PAR RE0074 recorder. All voltammetric measurements were made using a conventional three electrode design and at room temperature (20 ± 1°C). The reference electrode was a saturated calomel electrode (SCE), and a platinum wire served as the auxiliary electrode. The working electrode was a carbon paste prepared by pressing the paste into the well of the Teflon body of a home-made electrode (2 mm depth, 3 mm diameter). The carbon paste was from Metrohm (A.G.) Switzerland and prepared from spectroscopic grade graphite and UVASOL liquid paraffin (Metrohm EA 207 C). Controlled potential coulometry was performed on a PAR potentiostat, Model 173, and a PAR digital coulometer, Model 174, in a three electrode cell, Model 9660, containing a cylindrical platinum gauze working electrode (3.6 cm diameter, 20 cm height). The entire process of the electrolysis was followed by cyclic voltammetry at the CPE as a function of time. The pH values were measured with a Tacussel 60 pH-meter.

Reagents and solutions

All reagents were of analytical grade. Tizanidine hydrochloride (Sirdulad®) was generously provided by Sandoz (Brussels). The molecules 2,1,3-benzothiadiazole (II), 4-amino-2,1,3-benzothiadiazole (III) and 1,2-phenylenediamine (IV) were from Janssen Chimica (Geel, Belgium) and were used without further purification. Stock solutions of the compounds were prepared by dissolution in methanol. Buffer solutions (0.1 M) were prepared from disodium monohydrogenophosphate and the pH was adjusted with NaOH or HCl solutions. The analysed solutions contained 10% methanol. Deionized water was used. When necessary (reduction) dissolved oxygen was removed from the analysed solution by passing purified nitrogen into the cell for 10 min.

Results and Discussion

Electrochemical oxidation

Cyclic voltammetry conducted at the CPE on 1×10^{-4} M solutions of tizanidine (scan rate 50 mV s^{-1}) showed on the positive potential scan direction, and over the pH range investigated, a single oxidation peak close to the solvent oxidation (Fig. 2, curve a). The evolution of the peak potential (Epa) as a function of pH showed two linear segments (Fig. 3). By increasing the pH, the oxidation

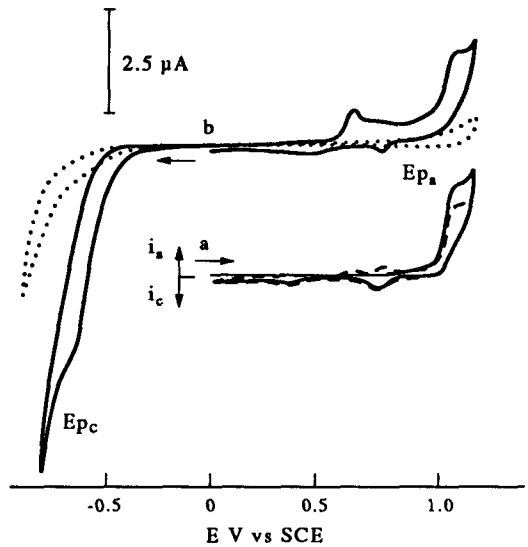

Figure 2
Cyclic voltammograms of tizanidine at the CPE in H_2SO_4 (0.1 M) + 10% methanol. Scan rate = 50 mV s^{-1}. (a) Positive potential scan direction, (b) negative potential scan direction. Dotted line = supporting electrolyte, dashed line = second scan.

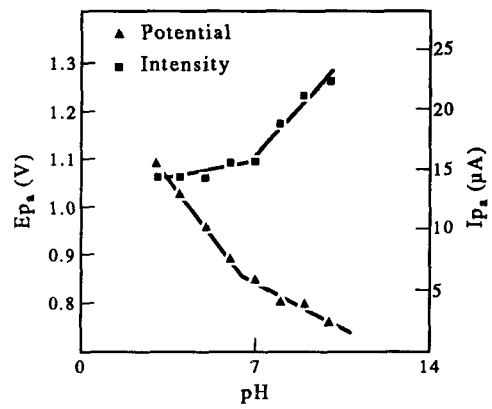

Figure 3
Evolution of tizanidine peak potential (▼) and intensity (■) as a function of pH at the CPE (scan rate 50 mV s^{-1}).

occurred at less positive potentials, the peak (Epa) shifting by 67 mV pH^{-1} up to pH 7, then by 28 mV pH^{-1} up to pH 10. The peak current intensity remained quasi-constant till pH 7 then increased linearly up to pH 10 (Fig. 3). Cyclic voltammograms recorded as a function of scan rate at pH 3 and 10 suggested an oxidation process controlled by diffusion (linear relationship between I_p and $v^{1/2}$) and irreversible within the scan rates 5–500 mV s^{-1} as evidenced by the absence of a corresponding cathodic peak on the reverse scan. Continued cycling of tizanidine revealed the appearance of slight and closely spaced reversible peaks in a less positive potential region. These peaks were observed in acidic media but their intensity decreased gradually by raising the pH and were not detected above pH 7. The presence of these redox couples located at less positive potentials than the original drug suggested that a chemical reaction subsequent to Epa occurred, giving rise to molecules more readily oxidized than tizanidine. The number and relatively low intensity of these peaks suggests a complex nature of the oxidation process of tizanidine. Controlled coulometric experiments were performed at potentials corresponding to Epa oxidation at pH 3 (E_{app} = +1.15 V) and at pH 10 (E_{app} = + 0.85 V). By following the electrolysis by cyclic voltammetry at the CPE it was observed that at both pH values the oxidation of tizanidine was not completed. This may be due to the close proximity of tizanidine and solvent oxidation potentials. At pH 3, a 50% decrease of Epa intensity corresponded to an overall transfer of two electrons. An exhaustively electrolysed solution at pH 3 was a red colour and cyclic voltammograms (CPE) of the solution showed that four reversible redox couples resulted from the oxidation of tizanidine. Electrolysis performed at basic pH values were unsuccessful due to the concomitant solvent oxidation at the platinum electrode.

Quantitative measurements made at pH 10 (highest electrode response) using differential pulse voltammetry (scan rate 5 mV s^{-1}, pulse frequency 1 s^{-1}, potential amplitude 50 mV) showed that the CPE response was linearly related to the concentration of tizanidine within the range 2×10^{-5} M–1×10^{-4} M and obeyed the equation: $I_p(\mu A) = 0.141 + 0.016\ C\ (10^{-5}\ M)$ with a correlation coefficient of 0.9991. The rather high limit of quantification (1×10^{-5} M) may be explained by the poor resolution of Epa and the solvent oxidation potential.

From these results it may be inferred that distinct oxidation mechanisms occurred depending on the protonation of the molecule; pK_a of tizanidine = 7.4 [7]. In order to identify the site where the primary oxidation occurred, cyclic voltammetry of tizanidine analogues, compounds II and III, have been carried out. Measurements were made in sulphuric acid (0.1 M) where the intensity and shape of the redox couples are better defined. Voltammograms of compound II showed no oxidation peak at the CPE. Cyclic voltammetry of compound III, however, showed that the molecule is readily oxidized in a single irreversible step at the CPE (Fig. 4). By reversing the potential-scan direction, new reversible and closely spaced peaks were observed at less positive potentials. Since compound II was not oxidized, and from the literature data on the electrooxidation of aniline in sulphuric acid media [8], it may be concluded that the oxidation of compound III occurred at the primary amine with possible formation of quinonic structures responsible for the redox couples observed at lower positive potentials. Likewise, it may be inferred that the oxidation of tizanidine occurred at the secondary amine nitrogen with possible formation of a quinoneimine structure (see redox couples in Fig. 2, curve a, and formulae in Fig. 5). It should be pointed out here, that the latter had not been detected but had been suspected as a possible metabolite in animals and man [5]. Additionally, the oxidation of the secondary amine might also proceed via liberation of the primary amine [8] which may be further oxi-

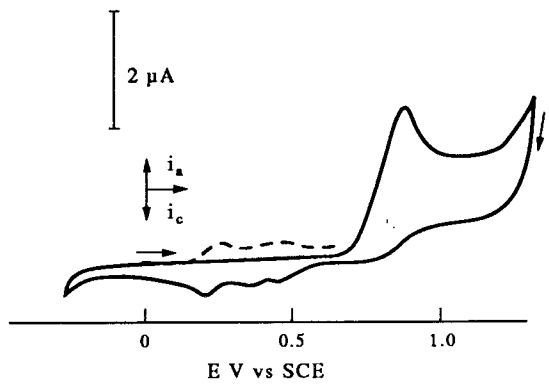

Figure 4
Cyclic voltammogram of compound III at the CPE in H_2SO_4 (0.1 M) + 10% methanol. Scan rate = 50 mV s^{-1}, dashed line = second cycle. Initial potential = 0.0 V vs SCE.

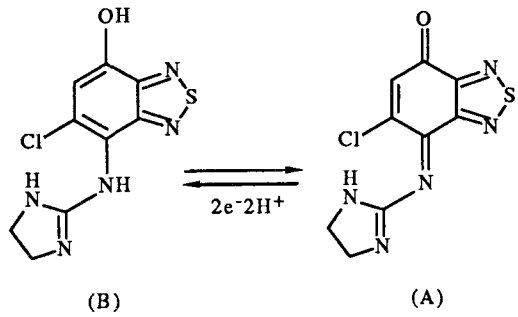

Figure 5
Structure of a possible oxidation product of tizanidine. (A) Quinone imine, (B) reduced form.

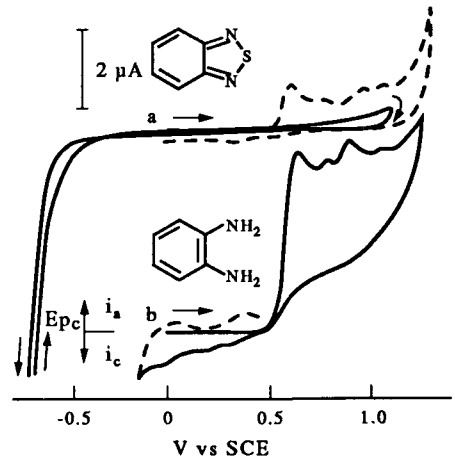

Figure 6
Cyclic voltammograms of compound II (a) and compound IV (b) at the CPE in H_2SO_4 (0.1 M) + 10% methanol. Scan rate = 50 mV s^{-1}. Initial potential = 0.0 V vs SCE. Dashed line = second cycle.

dized following the cyclic voltammetric behaviour of compound III (Fig. 4). The concomitant occurrence of these electrochemical reactions might explain the numerous redox couples detected in cyclic voltammetry and in controlled potential electrolysis.

Electrochemical reduction

Previous polarographic measurements have shown that tizanidine is irreversibly reduced in a single step over the range of pH 1–13, and that the mechanism corresponded to the opening of the cycle at the nitrogen–sulphur bond [equation (1)]. At the CPE, the reduction of tizanidine was observed close to the solvent reduction (Fig. 2, curve b). In the whole range of pH investigated, cyclic voltammograms showed one single irreversible peak (Epc). By reversing the potential scan direction, new closely spaced oxidation peaks were observed in the positive potential region between +0.5 and +1.0 V (Fig. 2, curve b). Subsequent cycling showed the quasi-reversible behaviour of these peaks. Following equation (1), these peaks seem likely to be related to the products of tizanidine reduction, i.e. H_2S and the diamine derivative. Since the former is not oxidized at the CPE, it was of interest to study the oxidation of a structurally related diamine, i.e. compound IV. Cyclic voltammograms were recorded in sulphuric acid media (0.1 M) and compared with the voltammograms of tizanidine and compound II. As shown in Fig. 6 curve a, the ortho-diamine structure was detectable at the CPE. The molecule was irreversibly oxidized with formation of new redox couples on subsequent scans (quinonic structures) [8]. The close matching of the peaks (number and potentials in the region +0.5 up to +1.1 V) of compound IV with the oxidation peaks appearing following reduction of compound II (Fig. 6, curve b) and tizanidine (Fig. 2, curve b) confirmed the mechanism given in equation (1).

Conclusions

The electrochemical study of tizanidine and structurally related molecules at the carbon paste electrode as a function of pH has permitted identification of the primary oxidation site and to postulate some possible oxidation products of tizanidine. It has been shown that the electrooxidation proceeds via formation of several oxidized quinonic structures which have been postulated in the literature as possible metabolites in animal and man. Cyclic voltammetry at the carbon paste electrode (reduction followed by oxidation) has allowed confirmation of the reduction mechanism postulated at the dropping mercury electrode. The analytical utility of the electrochemical detection mode in HPLC is limited by the high potential required for tizanidine oxidation. The detection and identification of the degradation products, however, would be ideally performed at carbon-based electrochemical detectors.

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