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ELECTROCHEMICAL STUDIES OF TIRAPAZAMINE: GENERATION OF THE ONE-ELECTRON REDUCTION PRODUCT

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The electrochemical properties of the benzotriazine di-*N*-oxide, tirapazamine (SR4233), and the mono- and zero-*N*-oxides, SR4317 and SR4330 respectively, have been investigated in dimethylformamide and acetonitrile. The voltammetry of tirapazamine is complicated, with up to 6 reduction steps being identified, depending on the solvent. Both SR4317 and SR4330 show two reduction steps. The first reduction of all three compounds is a reversible or quasi-reversible step, which is assigned to a 1-electron addition. Cyclic voltammetric studies show that the anion radical product is stable, although the tirapazamine 1-electron addition product shows a tendency to participate in a chemical following reaction. Subsequent reduction steps are all highly irreversible in nature. The 2nd electron transfer of SR4317 results in the formation of the free base, SR4330, which is identified voltammetrically. Comparison is made with the voltammetric behaviour of quinoline and quinoline-oxide.

KEY WORDS: Tirapazamine, benzotriazine-*N*-oxides, voltammetry, anion radical.

INTRODUCTION

Tirapazamine, 3-amino-1,2,4-benzotriazine-1,4-dioxide (SR4233), has entered phase I clinical trials as an agent for the treatment of solid tumours. The high selective toxicity of tirapazamine for hypoxic mammalian cells[1,2] involves reductive activation of the drug, which can only occur under conditions of low oxygen tension.[3] The short-lived reduction intermediate results in DNA damage and ultimately cell death. The proposed biologically active species is the one-electron reduction intermediate, as the two-electron product, the mono-*N*-oxide, SR4317, is biologically inactive.[4,5]

Our previous electrochemical studies on tirapazamine and a number of analogues, including SR4317 and the zero-*N*-oxide, SR4330, have used aqueous media.[6,7] Measurement of reduction potentials, the effect of pH, coulometry and bulk electrolytic reduction in the presence of DNA have been useful in assigning the reduction pathway. Tirapazamine can accept a total of six electrons but there is no evidence for the reduction process occurring in discrete one-electron steps *ie* the one electron species cannot be selectively generated and studied. In order to further extend our knowledge of the charge-transfer characteristics of tirapazamine we have turned our attention to the aprotic solvents, dimethylformamide and acetonitrile. To aid

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assignment of the voltammetry, the study has been extended to include the mono- and zero-*N*-oxide. Comparison is also made with the structurally related compounds the quinolines.

MATERIALS AND METHODS

Tirapazamine, SR4317 and SR4330 were obtained from Prof. J.M. Brown, Stanford University, California. Stock solutions of the compounds were prepared in dimethyl-sulphoxide (10 mM) and stored at −20 °C until required. Quinoline and quinoline-*N*-oxide were purchased from the Aldrich Chemical Co. All drugs and chemicals were used as received without further purification.

Electrochemical methods

Voltammetric experiments were carried out in dimethylformamide or acetonitrile (Aldrich Chemical Co., HPLC grade) with 0.1 M tetra-n-butylammonium perchlorate as the supporting electrolyte and purged with solvent saturated N_2. The typical concentration of the compound under investigation was 0.2 mM. Measurements were performed using a PAR 264A polarographic analyzer. All studies used a 3-electrode cell configuration, with a Pt wire counter electrode, and either Hg or Pt as the working electrode. For Hg, a PAR 303 cell stand was used, with an aqueous Ag/AgCl reference electrode. The dc polarographic studies used a dropping mercury electrode with electronically controlled drop-time (t_d) of 0.5 sec and a scan rate of 5 mV/s. Cyclic voltammetry (CV) used a hanging drop mercury electrode which was automatically renewed before each scan. The scan rate ranged from 10 to 500 mV/sec, but was routinely 100 mV/sec. With a Pt working electrode the reference was a non-aqueous Ag/Ag^+ electrode. Only the cyclic voltammetric behaviour was recorded.

RESULTS

Tirapazamine

The voltammetry is very different from that recorded previously in aqueous media.[5,6] In dimethylformamide at a mercury drop working electrode the response is complicated (Figure 1A) with up to 5 reduction steps being identified on the forward scan with peak potentials at $Ep_f = -1.05$, -1.38(shoulder), -1.48, -1.79 and -2.01 Volts. The 1st wave is well resolved, the 2nd and 3rd are minor processes and closely overlapping; the 4th and 5th are also overlapping but well formed with a current response comparable to the 1st reduction step. On the reverse potential sweep, only a single return wave is seen at $Ep_f = -1.40$ Volts. By variation of the switching potential this oxidation process can be assigned to the 4th and 5th reduction steps. On repeat cycling the 1st reduction wave is completely absent but a new reduction wave is seen at Ep_f −1.48 Volts, corresponding to the new oxidation wave seen on the return sweep (Figure 1A). Some shifts in the potentials of the 2nd and subsequent reduction steps are observed depending on the age of the drug solution, but the overall pattern of the voltammetry is unaltered. If the scan is reversed prior to the 2nd reduction step, then a clear return wave for

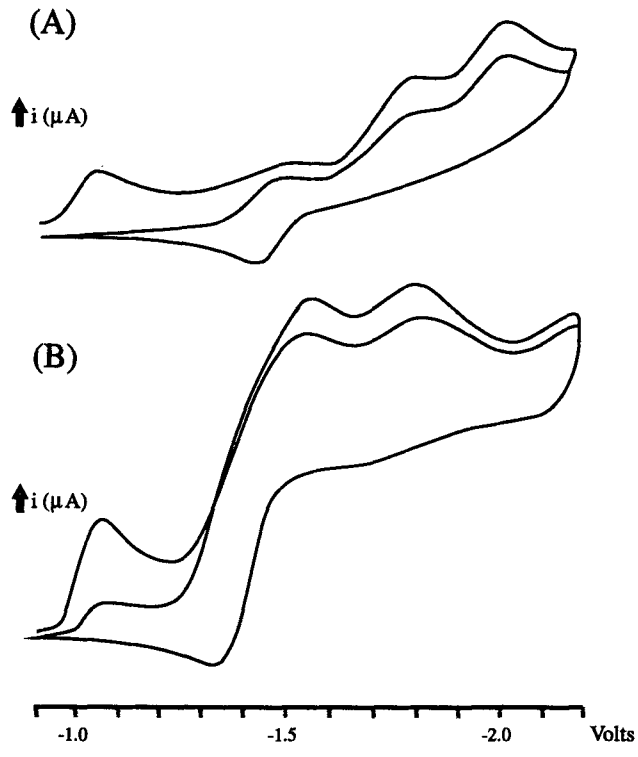

FIGURE 1 The cyclic voltammetry of tirapazamine (SR4233) showing the effect of repeat cycling. (A) in dimethylformamide; (B) in acetonitrile.

the 1st reduction step can be observed. Measurements on the first reduction step are listed in Table 1. The return wave develops further as the scan rate is increased. The concentration of the drug also influences the chemical reversibility of the 1st reduction step. Variation in the drug concentration between 0.05 and 0.74 mM resulted in a change in ip_r/ip_f from 0.90 initially to 0.45. At higher drug concentrations no return wave was found.

The dc polarography shows a well resolved wave for the 1st reduction. A logarithmic analysis gave a linear relationship with an $E_{½} = -1.025$ Volts. The more negative reduction steps were more poorly resolved and measurements were not attempted. The CV was also examined at a Pt working electrode. The complexity and general characteristics seen at Hg were repeated.

In acetonitrile the voltammetry is simplified. Three reduction steps are seen on the forward potential scan (Figure 1B) at $Ep_f = -1.08$, -1.58 and -1.83 Volts. The current response of the 2nd step is approximately twice that of the 1st. The 3rd is a relatively minor process. On the reverse potential scan a return wave is seen of comparable current response to the 2nd reduction. The peak potential depends on the switching potential used. Repeat cycling again shows a marked decrease in the forward current of the 1st reduction wave, but the 2nd and 3rd reduction steps, and associated return wave remain unaltered by repeat cycling. When only the 1st reduction is included in the potential window, distinct return wave character is found, the details of which are listed in Table 1.

TABLE 1
Cyclic voltammetric data on the first reduction step of tirapazamine (SR4233), SR4317 and SR4330

DRUG	*Dimethylformamide*				*acetonitrile*			
	$E_{½}$ Volts	ΔEp mV	ip_f μA	ip_r/ip_f	$E_{½}$ Volts	ΔEp mV	ip_f μA	ip_r/ip_f
SR4233	−1.025	55	0.60	0.58	−1.03	75	1.10	0.52
SR4317	−1.25	60	0.66	0.98	−1.29	60	1.08	0.92
SR4330	−1.345	80	0.65	0.61	−1.36	65	1.30	0.83
Q-oxide	−1.845	70	0.62	0.55				
Quinoline	−2.10[a]							

Recorded at a Hg working electrode at a scan rate of $\nu = 100$ mV/sec.
Potential measurements made with respect to a Ag/AgCl reference electrode.
[a] Potential at 80% of the forward wave current; return wave only observed at scan rates above 500 mV/sec.

SR4317

The voltammetry is very similar in both dimethylformamide and acetonitrile, irrespective of the working electrode. Details concerning the 1st reduction are shown in Figure 2A and are listed in Table 1. If the scan is extended to more negative potentials then a 2nd, very broad wave is seen, with approximate $Ep_f = -2.16$ Volts (Figure 2B). No return wave character was observed, but inclusion of this step results in the appearance of a cathodic shoulder on the 1st reduction wave, on both the return wave (which remains well developed) and on the second (repeat) negative scan. The dc polarography of the 1st reduction in dimethylformamide showed an uncomplicated wave. Logarithmic analysis gave a highly linear plot, with $E_{½} = -1.234$ Volts.

SR4330

In both solvents the voltammetric behaviour of SR4330 is very similar to that of SR4317, being essential independent of solvent and electrode material. Two reduction steps are seen. Details of the 1st are recorded in Table 1. The second reduction step is very distended in appearance with $Ep_f = -2.28$ Volts in dimethylformamide. No return wave was observed. Inclusion of this step did not influence the character of the 1st reduction. By dc polarography, the 1st reduction has no complicating features allowing a logarithmic analysis. An approximately linear relationship was found with $E_{½} = -1.325$ Volts.

DISCUSSION

The change from an aqueous to an aprotic solvent has a striking effect on the electrochemical behaviour of the three benzotriazine compounds examined, but particularly tirapazamine. The 1st reduction step of all three compounds has a number of features in common.

When the potential scan range is restricted to the first reduction, then considerable return wave character is observed (Table 1). The constant $E_{½}$, ΔEp and $ip_f/v^{½}$ values with changing scan rate are characteristic of a reversible, diffusion controlled charge-transfer step. The reduction product for SR4317 is highly stable (Table 1),

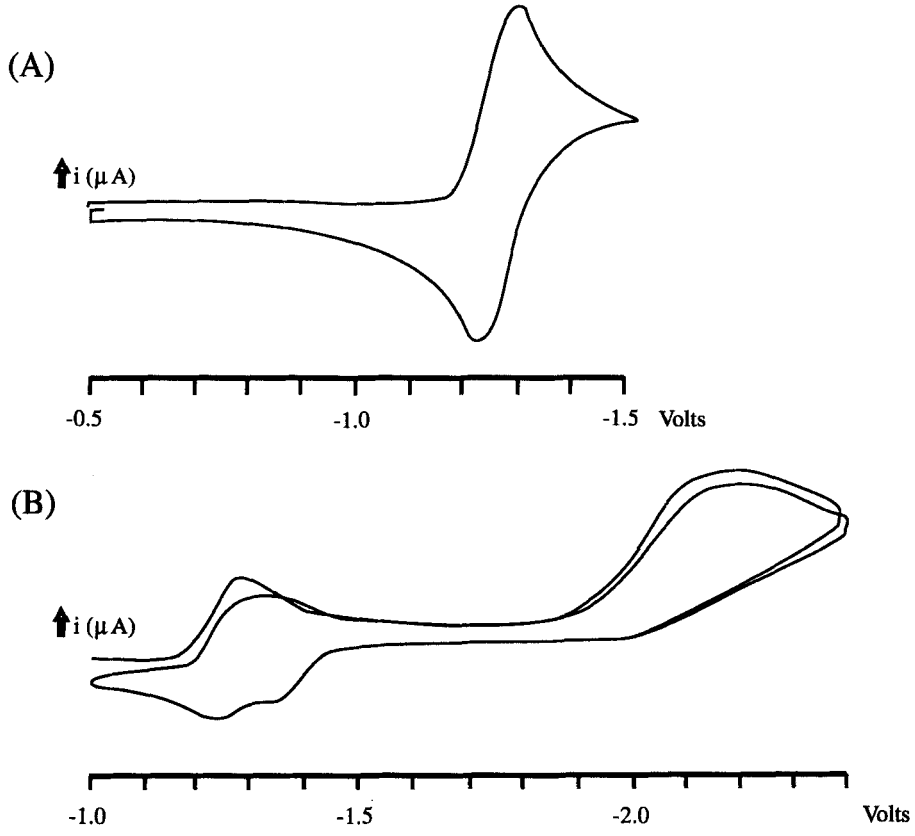

FIGURE 2 The cyclic voltammetry of SR4317 in dimethylformamide. (A) the first reduction step; (B) the complete potential range, showing the effect of repeat cycling.

with a return-to-forward peak current ratio (ip_r/ip_f) close to unity. The return wave for tirapazamine and SR4330 approaches unity as the scan rate is increased. This behaviour is in line with an irreversible chemical reaction following the reversible charge-transfer step. Drug concentration studies for tirapazamine showed that the stability of the 1st reduction product (as measured by the ip_r/ip_f ratio) was decreased as the drug concentration increased. This is indicative of the chemical following reaction being of second or higher order. This is in line with the proposed disproportionation of the tirapazamine 1-electron addition product.[8] Each of the compounds has ΔEp of approximately 60 mV which does not change with scan rate. This value is in line with a 1-electron addition step. The dc polarographic logarithmic analysis confirms the CV data that the electron transfer step is electrochemically reversible and diffusion controlled. Comparison of current responses for the three compounds by dc polarography and CV indicates that the same number of electrons is transferred in all cases.

Comparison can be made between the benzotriazine compounds and the related quinoline(Q) and quinoline-oxide(Q-oxide). In both classes of compound the electron affinity of the oxide is greater than that of the free base (Table 1). The 1st reduction step of the quinoline and quinoline-oxide compounds showed a reversible

CV response, which was also assigned to a 1-electron addition step. The stable anion radical product was characterized by ESR spectroscopy.[9,10,11]

The 2nd reduction steps of SR4317 and SR4330 are irreversible. For SR4317, inclusion results in a cathodic shoulder on the 1st reduction (Figure 2). This is assigned to formation of the zero-*N*-oxide SR4330. This is confirmed by addition of a genuine sample of SR4330 to the electrolytic cell. This behaviour is also in line with that found for the quinoline-oxides.[10,11]

The complete assignment of the voltammetry of SR4233 is not possible at this time. The CV response is clearly influenced by the solvent (Figure 1). The complexity observed in dimethylformamide is not duplicated in acetonitrile. In dimethylformamide, including the most negative reduction steps in the potential range results in the formation of a "new" redox couple, but despite similarities in potential and general appearance, this new couple does not correspond to either SR4317 or SR4330.

Further work is in progress to investigate more fully the electrochemical characteristics of the benzotriazines in aprotic solvents. Of particular interest will be the factors which influence the stability of the 1st reduction product of tirapazamine and the classification of the redox mechanism. Comparative studies on the quinoline and quinoline-oxides suggests that the presence of proton donors in the solvent will be of central importance.

In conclusion, the voltammetry of tirapazamine in dimethylformamide and acetonitrile exhibits a 1st reduction step which we have assigned to a 1-electron addition. This assignment is substantiated by comparison with quinoline and quinoline-oxides. The anion radical product has considerable stability showing a well developed return wave, although tirapazamine has a marked tendency to participate in following reactions. For the first time electrolytic techniques present the opportunity of specifically generating the proposed biologically active species for tirapazamine and studying its interaction with DNA.

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