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SHORT COMMUNICATION

Nitro Radical Anion Formation from Nitro-Substituted Amphetamine Derivatives

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ABSTRACT

The cyclic voltammetric characteristics of two nitroamphetamine derivatives (2-nitro-4,5-dimethoxyamphetamine and 2-nitro-4,5-methylenedioxyamphetamine) have been investigated in different media. In mixed media (aqueous buffer and DMF, dioxane, or acetonitrile) a reversible one-electron reduction takes place to form a stable nitro radical anion. At more negative potential values, a further three-electron reduction occurs irreversibly to give the hydroxylamine derivative. Cyclic voltammetry (CV) has been employed to study the tendency of the nitro radical anions to undergo further chemical reactions. The subsequent chemical reaction corresponds to a second-order process, a dismutation reaction electrochemically initiated. Data about rate constants and half-life times in mixed media are reported.

KEY WORDS: Cyclic voltammetry, Radical anion, Nitroamphetamine, Half-life time.

INTRODUCTION

Hallucinogens present a unique challenge to medicinal chemists who attempt the development of useful potency series, primarily because of limitations on the ability to gather clinical or meaningful in vivo data. For several years, attempts to elucidate structure-activity relationships and mechanism of action for hallucinogens have been carried out. These efforts have been directed toward the synthesis and evaluation of structural congeners and novel analogs of known hallucinogenic drugs [1,2]. The development of correlations between in vitro and in vivo data, based on series, can play an important role in identifying neuronal and receptor systems involved in the process of hallucinogenesis.

Phenylalkylamine derivatives have been demonstrated to interact with serotonin receptors of various peripheral tissue preparations [3,4] and have been also shown to interact with serotonin binding sites of rat brain homogenates [5]. Investigations of the structure-activity relationships of hallucinogenic drugs have been directed to the importance of the aromatic ring substitution in substituted amphetamine-type hallucinogens. The 2-nitro-4,5-dimethoxy and 2-nitro-methylenedioxy amphetamine derivatives are prototypes of this class of drugs. Some studies about their pharmacologic properties indicate serotoninergic effects [6].

Electrochemical data on phenylalkylamines are limited to a few articles [7,8]. Recently, our laboratory has reported [9] an interesting relationship between the electron-donating character of the C(4) substituent and the ease of oxidation of the ring. More recently, our laboratory has reported the cathodic behavior of the 4,5-dimethoxy-2-nitroamphetamine in aqueous media, mainly focused on its analytical determination [10].

Apparently, from the data reported in the literature up to date, the nitro group reduction is not directly involved in the pharmacological actions of this type of compounds. However, its presence in the molecule implies different physicochemical and electronic properties compared with those lacking this group.

Biotransformation pathways of nitroaromatic compounds are believed to result from nitroreductases that have been isolated in pure form and have the capacity to use nitro as either one- or two-electron acceptors. One-electron acceptance by the nitro compounds, which have the capacity to accept six electrons, results in the production of oxygen-reactive intermediates [11,12]. Two-electron acceptance by nitro compounds results in the production of extremely reactive nitroso intermediates. One-electron reduction of nitroso intermediates results

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in the production of oxygen-reactive hydroxylamine radical anions. In the case of the one-electron reduction product, the nitro radical anion, the reaction of this species with oxygen is usually faster than the ability of the enzyme to add a second electron; thus, further electron acceptance (reduction) of the nitro compound is inhibited [12]. This nitro radical anion becomes one of the most aggressive species in biological systems because of its both direct effect on endogenous molecules (DNA bases) and its well-known catalytic ability to transfer one electron to molecular oxygen with superoxide anion formation. This latter species has been involved in lipoperoxidative processes [13,14].

The aim of this communication is to determine the feasibility of nitro radical formation from these two nitro amphetamine derivatives through cyclic voltammetry (CV). In addition, this article provides information about the optimal conditions for the electrochemical generation of the anions and the kinetic characteristics of such species.

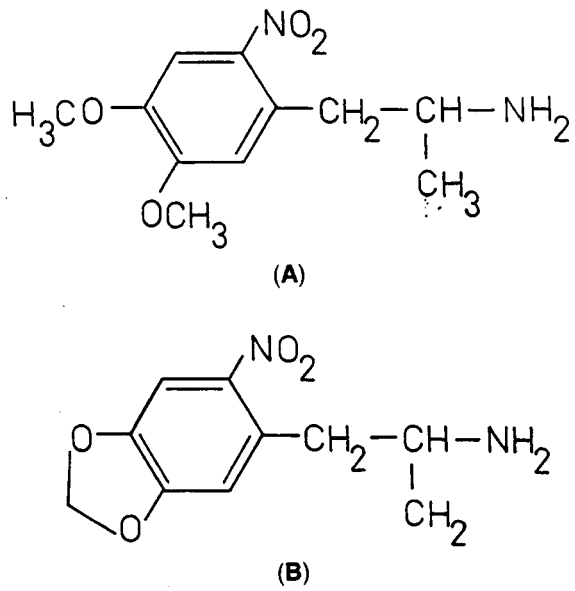

FIGURE 1. Chemical structures of 2-nitro-4,5-dimethoxyamphetamine (**A**) and 2-nitro-4,5-methylenedioxyamphetamine (**B**).

EXPERIMENTAL

Drugs

2-nitro-4,5-dimethoxyamphetamine (NDMA) and 2-nitro-4,5-methylenedioxyamphetamine (NMDA) were synthesized in a similar way as in a previous paper [10]. Dimethylformamide (DMF), acetonitrile, and dioxane were spectroscopic grade and were purchased from Merck.

Drug Solutions. Stock solutions were prepared in aqueous buffer, and then aliquots of the solutions were diluted containing different percentages of aprotic solvents (DMF, dioxane, or acetonitrile). The routine drug concentrations were maintained in 1.0 mM at all percentages of DMF. The influence of NDMA concentration was examined at % DMF = 50 and NMDA concentration at % DMF = 70, over a 0.05 to 1.0 mM range.

Buffer Systems

0.015 M trisodium citrate buffer containing various proportions of DMF was used as the electrochemical solvent (expressed as percent, v/v of DMF content). The ionic strength was kept constant at 0.3 M with KCl. Also, the following buffer systems were employed: 0.2 M phosphoric acid/0.2 M acetic acid; 0.2 M phosphoric acid/0.2 M carbonate; 0.1 M boric acid/0.1 M NaOH; 0.2 M glycine/0.2 M NaOH.

Cyclic Voltammetry

Electrochemical measurements were carried out with a Inelecsa assembly similar to the assembly described in a previous paper [15]. A Metrohm hanging mercury drop electrode with a drop surface of 1.39 mm^2 was used as the working electrode and a platinum wire as a counter electrode. All potentials were measured against a saturated calomel electrode.

All cyclic voltammograms were carried out at a constant temperature of 25°C, and the solutions were purged with pure nitrogen for 10 minutes before the voltammetric runs. At each DMF concentration, the return-to-forward peak current ratio, I_{pa}/I_{pc}, for the reversible first electron transfer (the Ar-NO_2/Ar-NO_2-couple) was measured, varying the scan rate from 0.05 Vs^{-1} up to 5.0 Vs^{-1}.

The experimental I_{pa}/I_{pc} ratios were calculated according to Nicholson's procedure, using individual cyclic voltammograms [16]. Furthermore, the switching potential, E_λ, was selected in order to minimize the influence of the second cathodic peak. Fifteen runs with E_λ varying between −900 and −970 mV versus SCE did not show a significant variation in the I_{pa}/I_{pc} values (coefficient of variation = 1.5%).

RESULTS AND DISCUSSION

The nitro amphetamine derivatives (2-nitro-4,5-dimethoxyamphetamine, [NDMA]) and (2-nitro-4,5-methylenedioxyamphetamine, [NMDA]) display an electrochemical response due to the nitro group reduction present in these molecules (Figure 1). This electrochemical response is strongly dependent on the nature of the media, distinguishing two different behaviors, that is, in aqueous and mixed media.

Aqueous Media

Cyclic voltammetry experiments for both nitro amphetamine derivatives in this medium show a similar behavior to that previously reported in an article concerned with NDMA [10]. Also, the present results are consistent with early reports for nitrobenzene [17,18] and nitroaromatic 1,4-dihydropyridines [15,19].

Mixed Media

Since the main goal of this study was to explore the feasibility of the nitro radical anion formation from these two derivatives, for the most part cyclic voltammograms were carried out in mixed media containing both aqueous and aprotic solvents.

In mixed media (aqueous citrate buffer/dimethylformamide, dioxane, or acetonitrile) both derivatives exhibit a substantial change in their reduction characteristics. The addition of different percentages of an aprotic solvent to aqueous solutions of these two derivatives makes it possible to separate the single four-electron reduction obtained in aqueous media to two different electron transfer processes. At physiological pH (7.4), it is possible to distinguish, first, a reversible one-electron reduction to form a stable nitro radical anion and second, an irreversible three-electron reduction process to yield the hydroxylamine derivative. Mixed media containing either different percentages of aprotic solvents (dimethylformamide, acetonitrile, dioxane) or buffer systems (phosphoric/acetic, phosphoric/carbonate, borax, glycine, citrate) were tested to evaluate the reduction mechanism of both derivatives. Results from these studies revealed that with all the tested aprotic solvents, the nitro radical anion formation is possible; however, the addition of citrate in the media is essential for the formation of the anion radical. Dimethylformamide was selected as the aprotic solvent, because of the best resolution and separation of both peaks. Figure 2 shows the cyclic voltammograms in the optimal mixed media conditions for the NDMA and NMDA derivatives (see the figure caption for the experimental conditions).

From these voltammograms, the following mechanism can be assumed:

$$\text{Ar-NO}_2 + e \underset{\text{(V)}}{\overset{\text{(IV)}}{\leftrightarrow}} \text{Ar-No}_2^{\cdot -} \qquad (1)$$

$$\text{Ar-NO}_2^{\cdot -} + 3e + 4\text{H}^+ \xrightarrow{\text{(I)}} \text{Ar-NHOH} + \text{H}_2\text{O} \qquad (2)$$

All the other peaks are the same as in aqueous media [10]. Through the selection of potential switching, it is possible to separate the peak due to the nitro radical anion.

In Figure 3, a typical voltammogram for the isolated $\text{Ar-NO}_2/\text{Ar-NO}_2^{\cdot -}$ couple for NMDA is shown. The electrode response analyses of this couple, for both derivatives, indicate that it is diffusion-controlled, involving one electron in the reduction process. In the scan range 0.05 to 5 V sec^{-1}, ΔE_p = 60 mV, $E_{1/2}$ is independent of the scan rate, I_{pa}/I_{pc} tends to unity, and I_{pc} versus $v^{1/2}$ is lineal. A similar mechanism in mixed media was previously reported in our laboratory for nitrendipine [15].

The chemical reversibility of the first reduction step, as determined by the I_{pa}/I_{pc} ratio in the cyclic voltammogram, increased with the addition of DMF, reaching a limiting value at approximately 50% for NDMA and 70% for NMDA. In spite of DMF stabilizing the nitro radical anion by limiting the protons in the media, the appearance of the monoelectronic reversible couple is dramatically affected by the nature of the buffer. Cyclic voltammograms of both derivatives performed in the optimal concentrations of DMF (50% for NDMA and 70% for NMDA) but in different buffer systems other than citrate, did not produce the reversible one-electron reduction. Simply, a poorly stabilized nitro radical anion was evidenced; that is, an $I_{pa}/I_{pc} \ll 1$ was found. Certainly, the effect of citrate is due to a surface phenomenon. It is possible that the citrate anion acts as a surface-active substance which displaces the radical anions from the

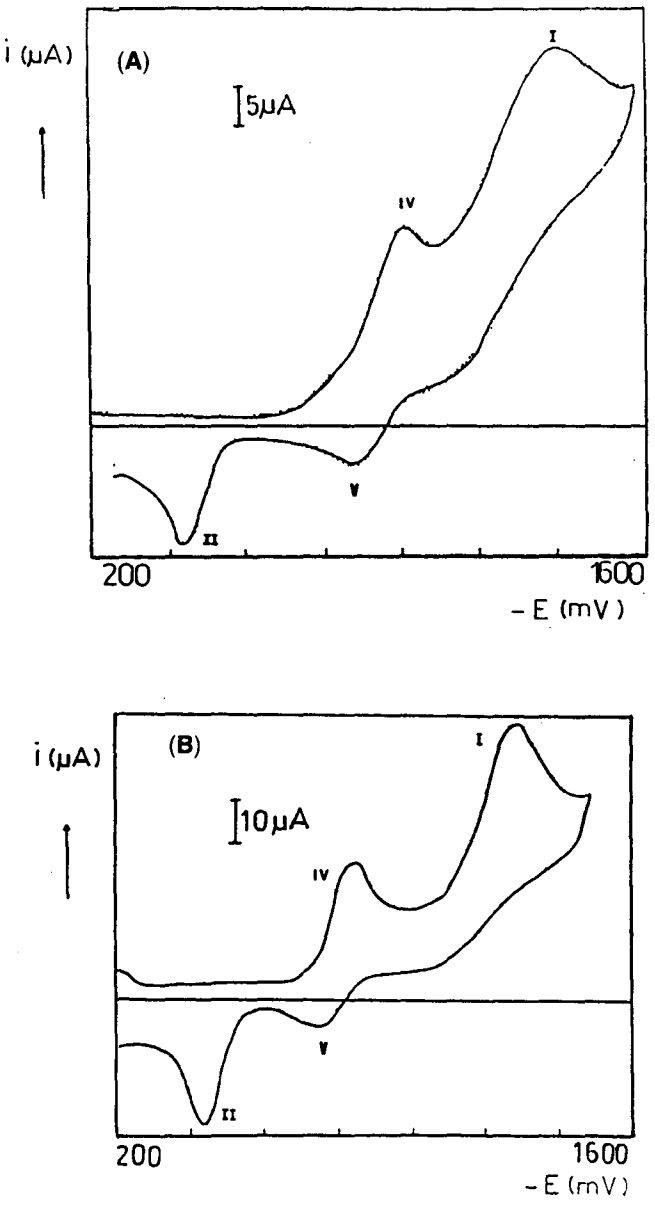

FIGURE 2. Cyclic voltammograms of the nitroamphetamine derivatives in the optimal mixed media conditions at 1 mM concentration. (**A**) NDMA in aqueous citrate buffer pH 10/DMF [50/50]. (**B**) NMDA in aqueous citrate buffer pH 10.5/DMF [30/70].

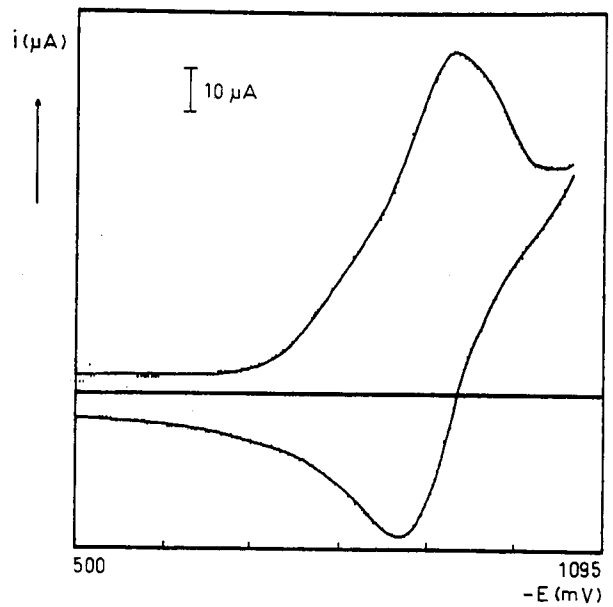

FIGURE 3. Cyclic voltammogram for the isolated Ar-NO_2/Ar-$NO_2^{\cdot-}$ couple of NMDA, in aqueous citrate buffer pH 10.5/DMF [30/70].

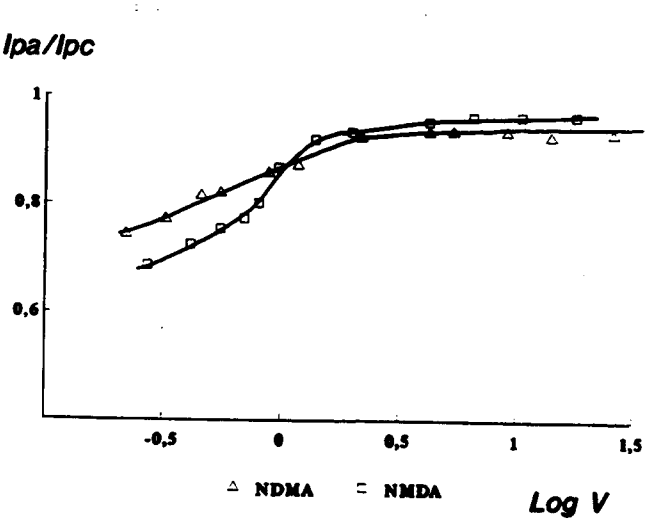

FIGURE 4. Dependence of the I_{pa}/I_{pc} ratio with the sweep rate for the nitroamphetamine derivatives.

electrode surface, thereby preventing their protonation at the surface; an effect which has been described for other types of substances [20,21].

The tendency of an electrochemically generated species to undergo chemical following reactions is reflected by the I_{pa}/I_{pc} ratio [22]. The coupled reaction does not equals unity but decreases if the reduction product reacts further; that is, a decline in the return wave occurs. Therefore, the CV mode can be used to probe the lifetime of the Ar-$NO_2^{\cdot-}$ species with changing electrochemical conditions, by measuring the I_{pa}/I_{pc} values of the Ar-NO_2/Ar-$NO_2^{\cdot-}$ couple.

To allow the RNO_2/RNO_2- couple to be examined in isolation, the switching potential (E_λ) was chosen at positive potentials relative to the second reduction step. Figure 4 shows the dependence between the I_{pa}/I_{pc} ratio and the scan rate for both derivatives. As can be seen, the ratio is different from the unity and depends on the scan rate. The current ratio increases as scan rate is increased. This result is in agreement with a chemical reaction following a reversible charge transfer, an EC_i mechanism. Supporting this mechanism is the finding that the cathodic peak potential depends on both the concentration of the amphetamine derivatives and the sweep rate. From the relationship dE_{pc}/d log C, we have obtained values of 20.3 mV for NDMA and 20.7 mV for NMDA, respectively. These results are in accord with the theoretical value of 19.5 mV for an EC_i process in which the chemical step follows a second-order kinetic. The fact that the current ratio does not reach the 1 value could be ascribed to a competition with a heterogeneous protonation.

On the other hand, to test the order of the following chemical reaction, the dependence between the I_{pa}/I_{pc} ratio and the concentration of the amphetamine derivatives was evaluated. The theory of cyclic voltammetry for a second-order reaction initiated electrochemically was exhaustively studied by Olmstead et al. [23]. According to that study, the current ratio will decrease as the concentration of electroactive species increases, because the second-order reaction is favored against the electrochemical reaction of the radical. Our results resemble the theoretical variation as predicted by Olmstead et al. for an E_rC_i second-order reaction (that is, a decrease of this ratio as concentration increases). In addition, the previously mentioned results and the relationship for dE_{pc}/d log C permit us to postulate the following general mechanism:

$$\text{Ar-NO}_2 + e \leftrightarrow \text{Ar-NO}_2^{\cdot-} \qquad (3)$$

$$2\,\text{Ar-NO}_2^{\cdot-} \xrightarrow{k_1} Z \qquad (4)$$

Based on results in which reduction schemes for nitro groups are discussed [17,24], presumably the second-order chemical reaction is the dismutation of the nitro radical anion. Furthermore, in Zuman and Fijalek's article [17] a competition between the homogeneous dismutation of the radical anion with a heterogeneous protonation, in order to form the protonated radical anion with immediately further reaction to the hydroxylamine derivative is asserted. In this study, the same competition is possible. According to the results presented in Figure 4, the obtained plateau at a ratio value different from 1 value is due to the effect of the heterogeneous protonation of the radical anion, and the increasing values of the I_{pa}/I_{pc} ratio at low sweep rates are due to the kinetic effect of the second-order dismutation reaction. Because the two competitive reactions occur on very different time schedules, the effects are perfectly distinguishable.

The second-order constants can be assessed from single cyclic voltammograms. Olmstead et al. [23] produced a working curve relating the I_{pa}/I_{pc} ratio with the kinetic parameter ω, defined by

$$\log \omega = \log k_2 c_0 \tau \quad (5)$$

This expression is valid when $a\tau = 4$, where k_2 is the second-order rate constant, C_0 is the nitro amphetamine concentration, and $\tau = [E_\lambda - E_{1/2}]/v$. The method can be applied under the experimental conditions here employed. Therefore, from the theoretical plot between the ratio I_{pa}/I_{pc} versus log $[k_2C_0\tau]$ at a known τ and C_0 (high enough to decrease the adsorption effect, in this case, 1×10^{-3} M) the value of k_2 can be obtained, although some contribution of the adsorption could be involved.

Supporting that the process followed Olmstead's theory (a second-order kinetic), plots of the kinetic parameter, ω, versus the time constant, τ, at different % DMF for both derivatives were found to be linear, with correlation coefficients of 0.991. Reproducibility for the method was tested for the I_{pa}/I_{pc} ratio and log k_2 by measuring ten independent runs for each nitro amphetamine derivative solution in the same experimental conditions (% DMF, pH, and sweep rate). For NDMA, average variation coefficients of 0.80% and 0.84%, and for NMDA, 0.81% and 0.74%, were obtained for the current ratio and log k_2 respectively. The half-life times for the nitro radical anion were also calculated assuming a second-order kinetic and at radical concentrations of 1×10^{-5} mol dm^{-3} [$t_{1/2}$(NDMA) = 18.6 seconds, $t_{1/2}$(NMDA) = 24.4 seconds at pH 10.5].

As expected, the rate constant decreased and half-life time increased as the % DMF increased. The absence of protons in the media favored the stability of nitro amphetamine radical anions. Plotting k_2 versus % DMF, linear relationships were found: k_2(NDMA) = -459 [DMF] + 28.7×10^3, correlation coefficient = 0.997; and k_2(NMDA) = -237 [DMF] + 20.5×10^3, correlation coefficient = 0.997. As expected, at higher DMF concentrations, nitro radical anions were more stable, and consequently, k_2 values were lower. The experimental average k_2 values at 50% DMF for NDMA (pH 10) and 70% DMF for NMDA (pH 10.5) were 5.4×10^3 dm^3 mol^{-1} sec^{-1} and 4.1×10^3 dm^3 mol^{-1} sec^{-1}, respectively.

CONCLUDING REMARKS

The present study shows that the CV technique and the experimental methodology here employed resulted in a useful tool to study redox intermediates. One of the major characteristics of the CV technique is the possibility of obtaining details not only on the reduction mechanism and the chemical stability of the reduction products but also on the influence of the environmental (solvent) conditions on kinetics of associated chemical reactions and the identity of the reduction intermediate(s). Such information provides guidelines to design new drugs with improved performance. On the other hand, the second half-life times calculated for the nitro radical anions electrochemically generated from nitro amphetamine derivatives permit us to conclude that these species are highly stable. Consequently, their therapeutic use as drugs or chemicals for semisynthesis for other type of compounds with different pharmacological activity is hampered by the formation of this type of toxic intermediate.

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