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644—TENTATIVE CORRELATION BETWEEN THE ELECTROCHEMICAL OXIDATION OF NEUROLEPTICS AND THEIR PHARMACOLOGICAL PROPERTIES *

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SUMMARY

A number of drugs are largely metabolized after oral administration, especially by redox processes. Among the neuroleptics, loxapine, clotiapine and clozapine, which are seven-membered tricyclic molecules with a piperazine side-chain, are known to be extensively metabolized by oxidation.

An electrochemical study of these compounds was initiated in order to determine their *in vitro* redox properties and to elucidate their oxidation mechanisms. The measurements were carried out in aqueous and non-aqueous media using voltammetric, cyclic voltammetric, coulometric, exhaustive electrolysis and thin-layer spectroelectrochemical techniques.

The oxidation mechanisms, which differ essentially depending on the pH of the solution, are suggested. In view of these results, various similarities have been detected between the *in vitro* oxidation processes and the pharmacological behaviour reported in the literature.

For example, the importance of the piperazine side-chain has been pointed out: oxidation no longer occurs if this side-chain is protonated; similarly, binding to the receptor is prevented if the lone electron pair of the tertiary atom is occupied. Nucleophilic additions have also been observed in non-aqueous media and the compounds have been identified using classical spectroscopic techniques. If the oxidation mechanism is identical on the piperazine chain for the three compounds, the process is somewhat different when it occurs on the tricyclic ring. Loxapine and clotiapine exhibit different behaviour from clozapine, in their electrochemical as well as in their pharmacological properties.

INTRODUCTION

Neuroleptic drugs, including phenothiazines, butyrophenones and related compounds, have proven to be extremely useful in the treatment of patients with severe psychiatric disorders. Among these drugs, clozapine, clotiapine and loxapine represent a distinct class of molecules which are structurally related to the phenothiazines. Their neuroleptic properties have been extensively studied, as reported in the literature [1–16].

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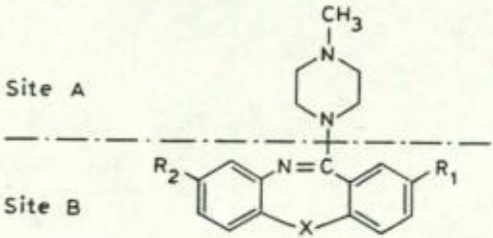
Structure–activity studies of these tricyclic neuroleptics (dibenzoazepines) have shown the importance of the nature of the basic side-chain for binding to the receptor. Moreover, the substitution in one of the benzene rings is essential for strong neuroleptic activity [10–16].

Although numerous pharmacological and biochemical tests have been done, a better understanding of how these drugs act in the brain could be attained by studying their *in vitro* redox behaviour. Indeed, like the majority of drugs, they are extensively metabolized, mainly by redox processes in the liver but also to a minor extent in extrahepatic tissues [3,5,6,16].

Electrochemistry represents an attractive technique which permits the generation and the identification of intermediates, and the elucidation of redox mechanisms.

The present paper presents a correlation between the *in vitro* oxidative properties of the three following molecules studied and their structure–activity relationships.



	X	R_1	R_2
Clozapine	N–H	H	Cl
Clotiapine	S	Cl	H
Loxapine	O	Cl	H

EXPERIMENTAL

Clozapine, clotiapine, clotiapine sulfoxide and clotiapine-sulfone were obtained from Wander (Switzerland). Loxapine was obtained from Cyanamid Benelux (Brussels). These compounds and reagent-grade buffers (phosphate buffer) were used without further purification. Stock solutions of the drugs (10^{-3} *M*) were prepared fresh daily by dissolving directly in 0.1 *M* sulfuric acid or in acetonitrile.

Polarographic *a.c.*, *d.c.* and d.p.p. experiments were carried out in aqueous media with a PRG3 and PRG4 Tacussel polarograph, connected to a PRT 30-01 potentiostat equipped with a three-electrode system and an EPL2 X–Y recorder.

Electrochemical oxidation was carried out in aqueous and non-aqueous media.

Voltammetric measurements (*d.c.*, d.p.p.) in aqueous media were made on a Bruker E100 polarograph with a Hewlett-Packard 7004B recorder. A three-electrode cell was thermostated at 25.0 ± 0.2 °C. The working electrodes were: a carbon paste Metrohm EA 267 prepared from a standard paste Metrohm EA 267C, a glassy carbon Metrohm AG and a platinum Tacussel EDI.

Non-aqueous voltammetric measurements were taken in acetonitrile in the presence of 0.1 *M* lithium perchlorate using PAR 175 and 176 instruments with an X–Y Sefram TGM 164 recorder. The three-electrode Tacussel RM04 cell contained a Tacussel EDI platinum working electrode, a platinum counter-electrode and an $Ag|10^{-2}\ M\ Ag^+$ reference electrode.

Cyclic voltammetric measurements were recorded on a PAR 175 instrument.

Coulometry and exhaustive electrolysis were carried out using PAR 173 and 179 instruments with a 9,600 cell.

The equipment for thin-layer spectroelectrochemistry has been described elsewhere [17–21].

U.V. and visible spectroscopy were carried out on a Beckman DB-T spectrophotometer, I.R. spectroscopy on a Beckman 4240, and mass spectrometry on an AEI-MS50.

The electrochemical exhaustive electrolysis in non-aqueous media and the perchlorate of the different phosphonium cations were realized and the products were detected and identified by thin-layer chromatography, I.R., U.V., visible and mass spectrometric techniques as reported earlier [19].

RESULTS AND DISCUSSION

The electrochemical oxidation [17,19] and reduction [18,20] of the molecules have been studied quite extensively. Table 1 contains a summary of the mechanisms proposed.

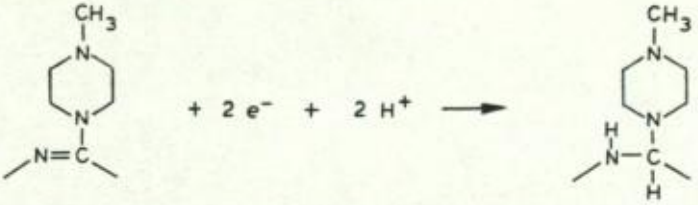
Reduction

The behaviour of the three compounds was similar. The polarographic (*d.c.*, *a.c.*, d.p.p.) and coulometric measurements have been used to demonstrate that the reduction occurs on the azomethine function situated in positions 10–11, each

TABLE 1

Mechanisms of reduction and oxidation

Reduction



Oxidation

	When piperazine site is protonated		When piperazine site is not protonated	
	Aqueous	Non-aqueous	Aqueous	Non-aqueous
Clozapine	Dimerization	Dimerization		
Clotiapine	Sulfoxidation and sulfonation	Not oxidizable	Similar behaviour for the three compounds: formation of an unstable iminium cation	
Loxapine	Not identified	Not oxidizable		

compound being reduced in a two-electron step. This process is influenced by the piperazine side-chain, due to the protonation of the proximal nitrogen of this group. The reaction appears to be irreversible in the whole range of pH investigated [18.20].

Oxidation

The oxidation has been realized in aqueous [17] and non-aqueous media [19]. The results suggest that the three molecules possess two electroactive sites: A and B. When site A is protonated, the oxidation occurs on the tricyclic ring (B). and the resulting oxidation products are quite different depending on the type of molecule studied and the nature of solvent used.

When the side-chain is protonated

Non-aqueous media. Cyclic voltammetric measurements carried out in acetonitrile in the presence of two equivalents of perchloric acid indicated that when the piperazine side-chain is di-protonated, oxidation no longer occurs on this part of the molecule. Under these conditions, clotiapine and loxapine are not oxidizable. Clozapine is still oxidized, but the reaction is located on the tricyclic ring (Fig. 1; peak Ep_{a3}). In this case, a dimerization process occurs giving rise to a dimeric structure which is more easily oxidized than the parent compound.

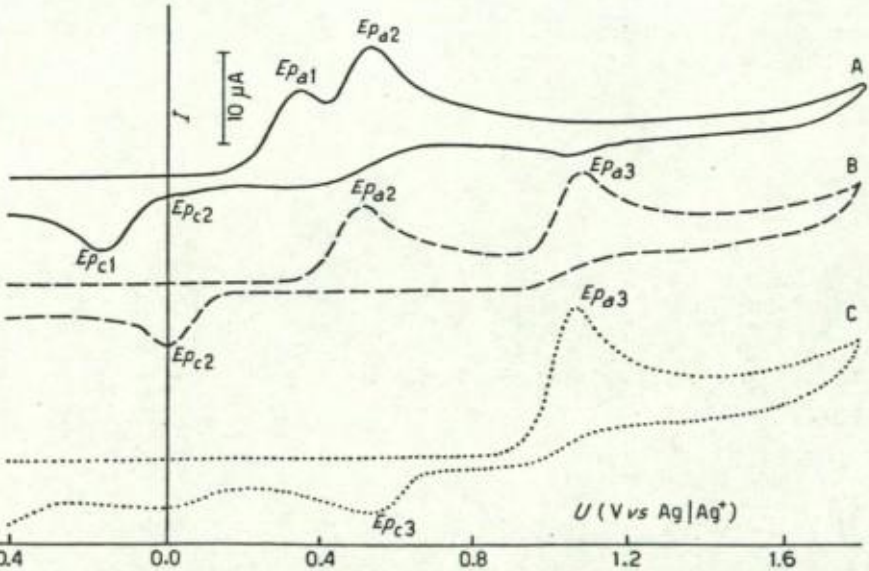
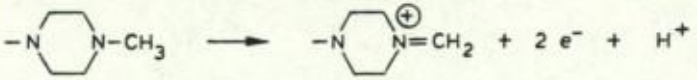


Fig. 1. Cyclic voltammograms at a stationary platinum electrode; clozapine, 1×10^{-3} *M*. (A) Acetonitrile + 0.1 *M* $LiClO_4$; (B) acetonitrile + 0.1 *M* $LiClO_4$ + 1 equivalent of $HClO_4$; (C) acetonitrile + 0.1 *M* $LiClO_4$ + 2 equivalents of $HClO_4$.

Aqueous media. The oxidation also occurs on the tricyclic ring (site B). Clozapine is reversibly oxidized to a dimeric structure as in non-aqueous media. In contrast to the non-aqueous media, clotiapine and loxapine still remain oxidizable. For clotiapine, the process is irreversible, giving rise to the corresponding sulfoxide and sulfone. Nevertheless, the yields are low due to the concomitant solvent oxidation. Loxapine is also irreversibly oxidized; the process occurs on site B, but identification of the products is difficult due to the rapid solvent oxidation and surface phenomena.

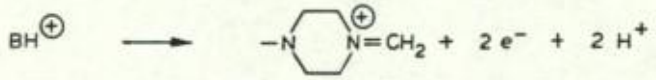
When the side-chain is not protonated

In view of the pK_a of these molecules ($pK_{a1} = 7.5 \pm 0.3$; pK_{a2} between 2 and 5), such a situation corresponds to the physiological pH. The three compounds exhibited similar behaviour when oxidized at solid electrodes in aqueous and non-aqueous media [19] (Fig. 1, curve A, peak Ep_{a1}). The oxidations lead irreversibly to the formation of an iminium cation:

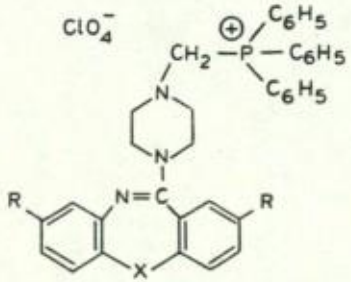


B iminium cation

When the piperazine site is mono-protonated, the molecules are still oxidizable but at more positive potentials (Fig. 1, curve B, peak Ep_{a2}):



In water, the iminium cation is rapidly hydrolysed, but in the presence of acetonitrile with an excess of a nucleophilic agent (triphenylphosphine), the cation forms a stable addition product, which can be easily isolated and identified:



DISCUSSION

In contrast to the oxidation of the phenothiazine nucleus, the oxidation of clotiapine, loxapine or clozapine does not give rise to any observable cation radical in the cyclic voltammetric measurements in both aqueous and non-aqueous media. This difference is of primary importance since positive radical ions play an essential part in the mechanism of psychotropic activity of the phenothiazine drugs [22]. Moreover, these highly stable, phenothiazine-free radicals are known to produce adverse effects, since they bind covalently and irreversibly with protein amino-acid residues, resulting in protein alteration [23].

However, in the case of the oxidation of the three drugs investigated, an electrophilic structure is also suggested, but this iminium cation is highly unstable. The reaction with nucleophiles is reversible, and the addition products are stable only in non-aqueous media since in the presence of water they are rapidly hydrolysed to the parent compound.

These observations could in part explain the relative low toxicity of clozapine, clotiapine and loxapine compared to the phenothiazines.

Structure–activity studies reported in the literature have pointed out two distinct pharmacological sites on the molecules investigated (sites A and B) [10,15]. Due to the analogy of these sites with the electrochemical one, it is of interest to present a detailed comparison between the pharmacological and the electrochemical sites. Table 2 illustrates a direct correlation between the electrooxidative and the pharmacological sites.

Pharmacologically, the nature of site A is of great importance for binding to receptors. For optimal neuroleptic activity, the distal nitrogen must be tertiary and

TABLE 2

Structure–activity relationship

Electrochemical. Two electrooxidative sites: A and B	Pharmacological. Two active sites: A and B
Site A	
Oxidation behaviour similar for the three molecules	Essential for neuroleptic activity for the three drugs
Importance of the distal nitrogen: –if protonated, oxidation is more difficult –if demethylated, –no iminium cation, –no nucleophilic addition	Distal nitrogen must be tertiary for binding to the receptors: –if protonated: no binding –if demethylated: loss of activity
Site B	
Oxidation behaviour similar for clotiapine and loxapine	Aromatic substitution essential for strong activity
Clozapine is easily oxidized	Clotiapine and loxapine analogous

the lone pair of electrons on the nitrogen must be unoccupied, otherwise binding to the receptor would be prevented [15].

Electrochemically, the results also suggest the importance of the piperazine side-chain. When the lone pair of electrons is protonated, oxidation is more difficult; if both nitrogens are protonated, oxidation no longer occurs on this site. Moreover, the distal nitrogen must be tertiary, otherwise no iminium cation and no addition product are obtained.

These findings, coupled with the fact that precise and complete knowledge of the receptor binding mechanisms for these molecules is at present not available in the literature [15], suggest that oxidation mechanisms in the brain, giving rise to reversible or irreversible bonds, could play an important role in the pharmacological activity of the drug.

Strong neuroleptic activity requires a nuclear substituent on the tricyclic ring (site B). Clotiapine and loxapine exhibited both similar electrochemical and pharmacological behaviour. Clozapine behaves differently from the other two. Oxidation on site B occurs at lower potentials than for clotiapine and loxapine, and the resulting products differ markedly.

Finally, it is noteworthy to establish that the oxidative sites pointed out during our electrochemical studies constitute the same *in vivo* oxidative sites as those suggested by the chemical structures of the metabolites detected in humans [3,5–7].

CONCLUSION

Electrochemistry applied to neuroleptics has permitted the elucidation of *in vitro* redox processes and, to some extent, has suggested that there is a close correlation between electrooxidative sites and the structure–activity relationship.

Even if it is not possible to demonstrate a direct causal relation, such an approach could provide an appropriate impulse for a better understanding of the *in vivo* behaviour of these drugs.

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