ticipated results. In contrast to the ointment, where a plateau drug level of 30–40 ng/ml was achieved, both gels (three animals in each trial) gave negligible (<1 ng/ml) plasma nitroglycerin concentrations over 4 hr. The *in vivo* absorption data did not correlate with the *in vitro* release results.

The composite data suggest a specific skin–vehicle interaction with the ointment, which has the net effect of increasing nitroglycerin permeability or decreasing the effective skin barrier thickness. Alternatively, poor nitroglycerin absorption from the neat material, alcoholic solution, and polyethylene glycol gels may have arisen from unfavorable interactions with the skin. A recent study showed that benzocaine diffusion through human stratum corneum decreased in the presence of relatively high amounts of low molecular weight polyethylene glycol (23). These researchers also showed that polyethylene glycol significantly affected the surface structure of the stratum corneum. These effects may be tested by comparing the absorption of a marker chemical following application of neat nitroglycerin or polyethylene glycol gels on the skin to absorption through untreated skin.

The ointment may increase transdermal nitroglycerin delivery by inhibiting skin metabolism. Enzymatic process in the skin and considerations of the skin as an active metabolizing barrier were discussed recently (24, 25). Previous studies also showed a total body nitroglycerin clearance from the rat in excess of reasonable hepatic clearance (26), suggesting extensive tissue degradation of this drug. The skin may represent a first-pass metabolic site for topically applied nitroglycerin. Interference with this process can affect the amount of intact nitroglycerin reaching the systemic circulation. This aspect of nitroglycerin transdermal delivery will be investigated.

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Kinetics of Dopamine Oxidation by Dialkylaminoalkylphenothiazine Cation Radicals

MARIA ROSA GASCO ˣ and MARIA EUGENIA CARLOTTI

Received March 28, 1978, from the *Istituto di Chimica Farmaceutica e Tossicologica dell'Universita' di Torino, Corso Raffaello 31, Torino, Italy.* Accepted for publication October 26, 1978.

Abstract □ The kinetics of dopamine oxidation by dialkylaminoalkylphenothiazine cation radicals (with two- or three-carbon side chains) were investigated. The two-carbon side-chain derivatives have reaction rates higher than the three-carbon ones. For chlorpromazine and promazine, extrapolation of pH 1–6 data shows that reaction rates become very fast at physiological pH.

Keyphrases □ Dopamine—oxidation by phenothiazine cation radicals, kinetics, structure–activity relationships □ Phenothiazine derivatives—oxidation of dopamine, kinetics, structure–activity relationships □ Structure–activity relationships—phenothiazine cation radicals, oxidation of dopamine, kinetics

Several reports (1–4) pointed out that transformation of chlorpromazine [2-chloro-*N*,*N*-dimethyl-10H-phenothiazine-10-propamine] (I) is necessary for some *in vitro* enzyme inhibitions. A I intermediate, the cation radical [obtained also *in vivo* (5)], was shown to inhibit (Na^+, K^+) adenosine triphosphatase (6). The cation radical also inhibited microsomal brain enzyme (7) and uridine diphosphate glucose dehydrogenase (8).

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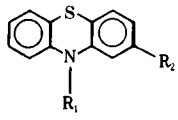
Table I—Structures of Investigated Compounds and Spectral Characteristics of the Corresponding Radicals



Compound	R_1	R_2	ϵ, $10^{-3}\ M^{-1}\ cm^{-1}$	λ_{max}
I Chlorpromazine[a] (2-chloro-*N,N*-dimethyl-10H-phenothiazine-10-propanamine)	$-CH_2CH_2CH_2N(CH_3)_2$	Cl	11.2 ± 0.5	523
II Promazine[a] (*N,N*-dimethyl-10H-phenothiazine-10-propanamine)	$-CH_2CH_2CH_2N(CH_3)_2$	H	9.2 ± 0.3	513
III Dietazine[a] (*N,N*-diethyl-10H-phenothiazine-10-ethanamine)	$-CH_2CH_2N(C_2H_5)_2$	H	10.5 ± 0.4	511
IV Parsidol[a] (*N,N*-diethyl-α-methyl-10H-phenothiazine-10-ethanamine)	$-CH_2CH(CH_3)N(C_2H_5)_2$	H	9.5 ± 0.3	515
V Promethazine[a] (*N,N,α*-trimethyl-10H-phenothiazine-10-ethanamine)	$-CH_2CH(CH_3)N(CH_3)_2$	H	9.3 ± 0.4	515
VI Isopromethazine[a] (*N,N,β*-trimethyl-10H-phenothiazine-10-ethanamine)	$-CH(CH_3)CH_2N(CH_3)_2$	H	8.8 ± 0.4	524

[a] Rhône-Poulenc, France.

Barras and Coult (9) observed that phenothiazine tranquillizers accelerated enzymatic biogenic amine oxidation while phenothiazines lacking clinical tranquillizing activity had no effect. Løvstad (10, 11), investigating ceruloplasmine-catalyzed phenothiazine oxidation, found that a three-carbon side chain was essential for a rapid enzymatic oxidation to the cation radical and for further oxidation by the same cation radicals of biogenic amines.

A previous paper reported the oxidation kinetics of tricyclic amines to the corresponding radicals with Ce(IV) (12); cation radical formation rates decreased while the reduction potentials increased, passing from the drugs with antidepressant action [imipramine (5H-dibenz[*b,f*]azepine-5-propanamine, 10,11-dihydro-*N,N*-dimethyl) and 10(9H)-acridinepropanamine, *N,N*,9,9-tetramethyl (dimetacrine)] to dialkylaminoalkylphenothiazines with a two-carbon side chain and then to dialkylaminoalkylphenothiazines with a three-carbon side chain.

In the present study, the reduction kinetics of phenothiazine cation radicals with different pharmacodynamic activities (Table I) by dopamine [1,2-benzenediol-4-(2-aminoethyl)] (VII) are investigated.

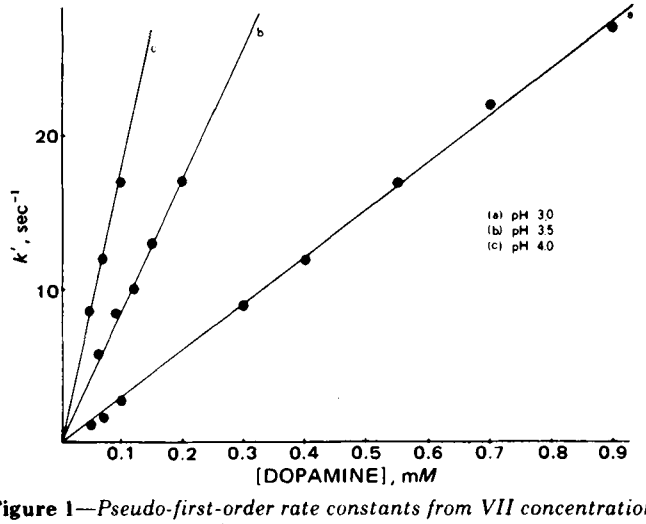


Figure 1—*Pseudo-first-order rate constants from VII concentration at different pH values (for II).*

EXPERIMENTAL

Reagents—The phenothiazine compounds (I–VI, Table I) were employed as chlorhydrates. Compound VII[1] and potassium exachloriridate were reagent grade.

Procedure—Cation radical solutions were obtained immediately before use either by adding potassium exachloriridate in slight deficiency to a solution of phenothiazine derivatives or by platinum electrode electrooxidation. Unoxidized phenothiazine had no effect on kinetics. The kinetic runs were carried out with a stopped-flow spectrophotometer[2] at the wavelength of maximum cation radical absorption (Table I). The cell path length was 2.00 cm.

Measurements were performed at different acidities: the pH ≤ 3 was obtained with perchloric acid, while the pH 3–6 range was obtained with potassium phthalate buffers. The pH measured by the potentiometer[3] was constant within 0.05 unit.

Pseudo-first-order conditions were chosen when possible; second-order conditions were adopted for the faster reactions. The cation radical concentration ranged between 5.0×10^{-6} and $5.0 \times 10^{-5}\ M$; the VII concentration ranged between 5.0×10^{-6} and $5.0 \times 10^{-4}\ M$.

The rate constants were evaluated by the least-squares method. Cation radical instability allowed a wide pH range investigation of I and II only; for other derivatives, only high acidity 0.1 *M* $HClO_4$ runs were performed. Since many radicals are unstable at high pH, the cation radical solutions were obtained at pH 3–6 with $1.0 \times 10^{-3}\ M$ $HClO_4$ and mixed with the solution of VII buffered at the desired pH. (The final pH variation was within 0.1 unit.) The cation radical decomposition rates, reported previously (13), were negligible compared to the ones in this study.

The initial cation radical concentration was estimated, when second-order conditions were used, by initial transmittance. Kinetic runs were carried out at 25° and, for I and II, at 35°.

[1] Dopamine, Merck, Schuchardt.
[2] Durrum-Gibson, Palo Alto, California.
[3] Polymetron 42/B.

Table II—Pseudo-First-Order Constants for Reaction between II Cation Radical and VII at 25° at Different Acidities[a]

VII, m*M*	pH 1.00[b]	2.00[b]	2.52[c]	3.00[d]	3.50[d]	4.00[d]	4.60[d]
0.050	—	—	—	1.2_5	—	8.6	—
0.060	—	—	—	—	5.8	—	33
0.070	—	—	—	1.8_5	—	12	—
0.090	—	—	—	—	8.4	—	58
0.100	—	—	—	2.8	—	17	—
0.120	—	—	—	—	10	—	74
0.15	—	—	—	—	13	—	—
0.20	—	—	—	—	17	—	—
0.30	—	4.7	6.2	9.0	—	—	—
0.40	2.18	—	—	12	—	—	—
0.50	3.40	7.8	8.8	—	—	—	—
0.55	—	—	—	17	—	—	—
0.70	4.30	10.5	12	22	—	—	—
0.90	—	13	—	27	—	—	—
1.00	—	—	17	—	—	—	—
1.50	—	—	25	—	—	—	—
5.00	27.0	—	—	—	—	—	—

[a] For pH 1.0–4.6, other kinetic runs were performed also in second-order conditions. For pH >4.60, the runs were performed in second-order conditions. [b] 5.0×10^{-6} *M* II cation radical. [c] 2.5×10^{-5} *M* II cation radical. [d] 5.0×10^{-5} *M* II cation radical.

RESULTS AND DISCUSSION

Stoichiometry—By adding successive VII aliquots to a cation radical solution in 0.1 *M* $HClO_4$, the decrease in absorbance at the proper wavelength was measured and the stoichiometric ratio was determined. The overall reaction can be depicted as:

$$2P^{+\cdot} + H_3D^+ \rightarrow 2P + DQ^+ + 2H^+$$

Scheme I

where P represents the phenothiazine derivative, $P^{+\cdot}$ is the corresponding cation radical, H_3D^+ is the fully protonated VII, and DQ^+ is the protonated dopamine quinone VIII.

Since the H_3D^+/DQ^+ reduction potential is ~0.78–0.79 v (14, 15), the reaction is shifted toward the right side with phenothiazine derivatives that exhibit higher reduction potentials or a higher pH. In the kinetic runs with less oxidizing cation radicals and excess VII, T_∞ transmittance at equilibrium came very close to 1.00. At pH 5, VIII can produce the corresponding aminochrome (16); however, the aminochrome formation rate is negligible compared to the reaction rates in this study (17).

Reaction Kinetics—In pseudo-first-order conditions, plots of $\ln(A_t - A_\infty)$ (where A_∞ and A_t represent the absorbance at equilibrium and at time t, respectively) as a function of time were linear for at least 80% of the reaction process (Table II).

The observed rate constants also exhibited a linear dependence on the VII concentration (Fig. 1). With second-order conditions, the related second-order plots were also satisfactorily linear. When the reaction rate

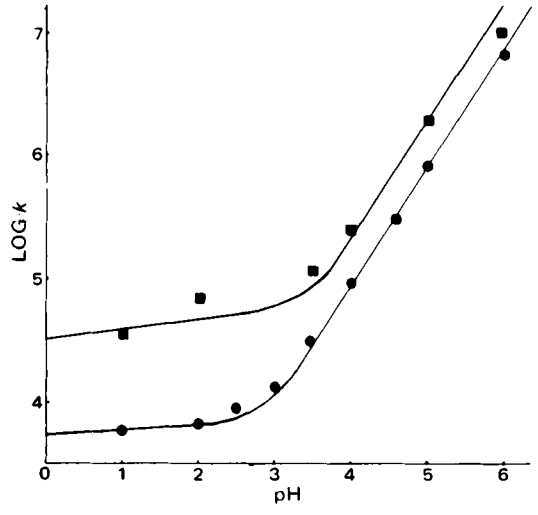


Figure 2—*The pH dependence of second-order rate constants, k, for VII oxidation with cation radicals (I and II) at 25°. The points (● for II and ■ for I) are experimental data. The curves were computed according to Eq. 2 with the values reported in Table III.*

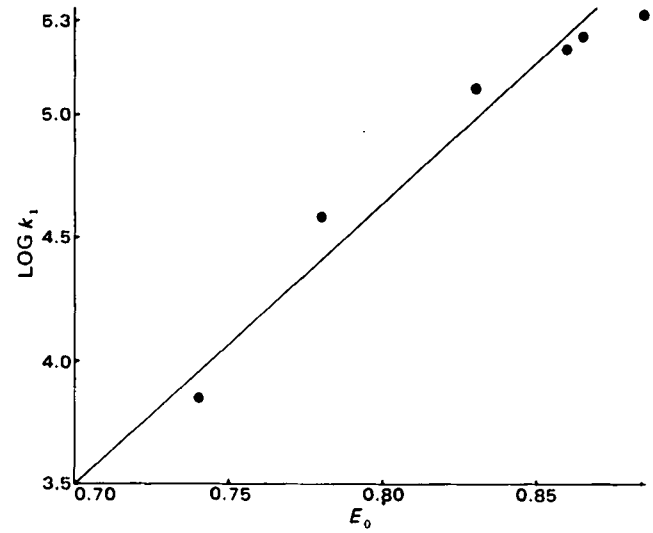


Figure 3—*Log* k_1 versus E_0 *plot of different phenothiazine cation radicals.*

was first order and dependent on each reagent concentration, the following rate law pertained:

$$-\frac{1d[P^{+\cdot}]}{2dt} = k[P^{+\cdot}][\text{dopamine}] \qquad \text{(Eq. 1)}$$

where [dopamine] represents the stoichiometric VII concentration. The observed rate constant values are reported in Table III.

pH Dependence—The data reported in Table III show that the rate constants were affected by the solution pH. This pH dependence can be explained by the different VII protonation states represented by:

$$H_3D^+ \underset{H^+}{\overset{K_1}{\rightleftharpoons}} H_2D \underset{H^+}{\overset{K_2}{\rightleftharpoons}} HD^-$$

Scheme II

where $pK_1 \cong 9$ and $pK_2 \cong 10$ (18).

Recent research (18) suggested that these dissociation constants represent competitive deprotonation and cannot be assigned to the ammonium or phenolic groups.

The acidity dependence can be ascribed to competitive paths such as:

$$P^{+\cdot} + H_3D^+ \xrightarrow{k_1} P + H_2D^{+\cdot} + H^+$$

Scheme III

Table III—Second-Order Constants for Reaction between VII and Different Phenothiazine Cation Radicals at 25°

pH	I	II	III	IV	V	VI
0.5	—	—	1.2×10^5	1.6×10^5	—	2.1×10^5
1.0	3.75×10^4	5.9×10^3	—	—	1.75×10^5	1.9×10^5
2.0	7.1×10^4	6.7×10^3	—	—	—	—
2.5	—	8.9×10^3	—	—	—	—
3.0	—	1.32×10^4	—	—	—	—
3.5	1.2×10^5	3.13×10^4	—	—	—	—
4.0	2.5×10^5	9.36×10^4	—	—	—	—
4.6	—	3.06×10^5	—	—	—	—
5.0	2×10^6	8.5×10^5	—	—	—	—
6.0	1×10^7	6.7×10^6	—	—	—	—

Table IV—VII Oxidation Rate Constants with Different Cation Radicals at 25°

Compound	E_0	k_1, M^{-1} sec^{-1}	k_2K_1, sec^{-1}
I	0.78	$(3.7 \pm 0.4) \times 10^4$	(20 ± 8)
II	0.72	$(6.0 \pm 0.8) \times 10^3$	(7.9 ± 0.9)
III	0.83	$(1.2 \pm 0.1) \times 10^5$	—
IV	0.86	$(1.6 \pm 0.2) \times 10^5$	—
V	0.86_5	$(1.75 \pm 0.2) \times 10^5$	—
VI	0.88_5	$(2.1 \pm 0.2) \times 10^5$	—

$$P^{+\cdot} + H_2D \xrightarrow{k_2} P + H_2D^{+\cdot}$$

Scheme IV

and then follows the fast reaction:

$$P^{+\cdot} + H_2D \rightarrow P + DQ^+ + H^+$$

Scheme V

This leads to the rate law:

$$-\frac{1d[P^{+\cdot}]}{2dt} = \frac{(k_1 + k_2K_1[H^+]^{-1})[\text{dopamine}][P^{+\cdot}]}{1 + k_1[H^+]^{-1}} \qquad \text{(Eq. 2)}$$

which with $K_1[H^+]^{-1} \ll 1$ in the pH range investigated reduced to:

$$-\frac{1d[P^{+\cdot}]}{2dt} = (k_1 + k_2K_1[H^+]^{-1})[\text{dopamine}][P^{+\cdot}] \qquad \text{(Eq. 3)}$$

Figure 2 compares the experimental points and a curve calculated with Eq. 3. The parameters k_1 and, when available, k_2K_1, were collected and were $\Delta H^{\neq} = 7.3 \pm 1.1$ kcal/mol and $\Delta S^{\neq} = -17 \pm 4$ cal deg^{-1} mole^{-1} for path I and $\Delta H^{\neq} = 4.8 \pm 1.3$ kcal/mole and $\Delta S^{\neq} = -38 \pm 4$ cal deg^{-1} mole^{-1} for path II. These values are overall data, including the contribution of dissociation constant K_1 and of the diffusion-controlled term.

CONCLUSIONS

For the observed reaction between a cation radical and an organic substrate, an electron-transfer mechanism can be proposed (19–21). When this mechanism is operating, a relationship between the reaction rates and the oxidizing agent reduction potentials is expected (22, 23). This provision is fulfilled for the acid-independent path. In fact, where the phenothiazine derivative reduction potentials are reported (12), the compounds with higher reduction potential exhibit the higher reaction rates (Table IV). This can be seen in Fig. 3, where a linear relationship between log k_1 and E_0 is shown. The acid-independent path, however, although investigated for only two compounds, shows a close rate, k_2K_1. [In fact, while the ratio of k_1(II)/k_1(I) is 6.2, the ratio of k_2(II)/k_2(I) is only 2.5.]

The values of the specific rate constant k_2 could be estimated by knowing K_1, for which the value around 10^{-9} mole/liter can be suggested (19–21). This procedure leads to k_2 values of 7.9×10^9 and 2.0×10^{10} liters mole/sec for I and II, respectively, which are very close to the diffusion-controlled limit (24).

When such conditions are reached, the contribution of the activation-controlled reaction can be expressed by (25):

$$\frac{1}{k_{obs}} = \frac{1}{k_{diff}} + \frac{1}{k_{act}} \qquad \text{(Eq. 4)}$$

Consequently, it should be possible, knowing k_{diff}, to derive k_{act}, the activation-controlled electron-transfer rate. If $k_{diff} = 3.0 \times 10^{10}$ liters/mole sec (24), it follows that $k_{act} = 1.0 \times 10^{10}$ and 6.0×10^{10} liters/mole sec for I and II, respectively. The ratio $k_{2act(II)}/k_{2act(I)}$ should be approximately equal to that observed for the ratio of an independent path. This suggests that the rate of path electron transfer is also related to the oxidizing species reduction potentials. Obviously, this agreement could be fortuitous, owing to the uncertainty on k_1 and k_{diff}. The observed rate constants become very fast at physiological pH. With these conditions, VII oxidation could be a way of disappearance of I and II cation radicals.

Since dialkylaminoalkylphenothiazines with two-carbon side chains have a higher reduction potential, their cation radicals show a quicker oxidation reaction than the ones with three-carbon side chains. There is, therefore, a correlation between VII oxidation rates and the side-chain structure. The higher cation radical oxidation potential of derivatives with two-carbon side chains could explain their different behavior (11) in enzymatic oxidation of VII. This parameter might also explain the difference in their pharmacodynamic activities.

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