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FREE RADICALS FROM IMIPRAMINE

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Abstract—A blue chromophore is produced from imipramine by anodic electrolysis and by reaction with ceric ion or other univalent oxidizing systems. Paramagnetic resonance confirms the free radical character of the product, which is metastable in solution but is stabilized in strong acid. The possible relationship of the free radical to the mechanism of drug action is discussed on the basis of qualitative similarities between the pharmacology of imipramine and of phenothiazines, which are known to form semiquinones under comparable conditions.

THE differences between imipramine and the phenothiazine drugs appear to be largely quantitative. This has been confirmed by biochemical tests *in vitro*[1–5] and by pharmacological studies in animals.[6–9] *In vivo*, impramine and the phenothiazines manifest a wide spectrum of generally "depressant" activities,[9,10] yet both imipramine and chlorpromazine display some "analeptic" properties at lower doses.[9] These similar effects have led to the suggestion that the "mediator nature" and possibly the loci of action of imipramine and of chlorpromazine are common.[9] Previously we showed that metastable semiquinone radical ions could be formed from chlorpromazine congeners *in vitro*[11] under mild reaction conditions.[12] This communication reports on free radicals formed similarly from imipramine.

EXPERIMENTAL

Imipramine hydrochloride (3-dimethylaminopropyl-dibenzyl amine) was a gift from Geigy Pharmaceuticals. It was > 99% "pure" and was used as supplied. Other chemicals were reagent grade.

Optical absorption spectra were recorded with a Beckman DK-2 ratio-recording double-beam spectrophotometer. Polarography was done by means of Sargent model XV polarograph.

Potentiometric titrations were carried out in 5-ml volumes, with a Beckman Zeromatic pH meter equipped with a platinum microelectrode and a saturated calomel reference electrode. Solutions to be titrated were kept under nitrogen and were stirred with a magnetic stirrer. In some experiments potentials were recorded as a function of time with a Varicord model 43 recorder (Photovolt Corp.). Increments of concentrated oxidant were added from a syringe-type microburet (Micro-Metric Instrument Co.) driven by a clock motor during only part of a repeated time cycle, which was set by an adjustable microswitch and a second clock motor. This apparatus permitted intermittent tiration so that irreversible reactions might be detected by changes in the potentials recorded after each increment of oxidant (e.g. Fig. 3).

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Electron paramagnetic resonance (EPR) was effected with a Varian EPR spectrometer with 100 kc/s field modulation and a Fieldial magnetic field regulator. Continuous-flow and stopped-flow reactions were carried out with a flow system and special mixing cells that have been described previously.[13] A flat EPR cell containing a platinum foil anode (0·0005 inch thick) was used for electrolysis *intra muros*.[14] A working cathode isolated by a glass frit and a reference Ag/AgCl electrode were immersed in an electrolyte reservoir above the flat cell. The source of electric potential was a dry cell connected through a voltage divider; potentials were measured between the anode and the reference electrode with an electrostatic voltmeter (Kiethley Instruments, Inc.; model 210).

RESULTS

Colored products formed from chlorpromazine and its congeners by the action of several transition-group metal ions of biological importance[12] and by reaction with ceric ion[11] have been described. These chromophores were identified as semiquinone radical ions.[11] Imipramine hydrochloride reacts similarly: titration to pH $\sim$ 7 with NaOH followed by back titration with HCl of a solution 0·005 M in both imipramine and $MnCl_2$[12] converts the initially colorless solution to pale tourquoise-green. Ferric

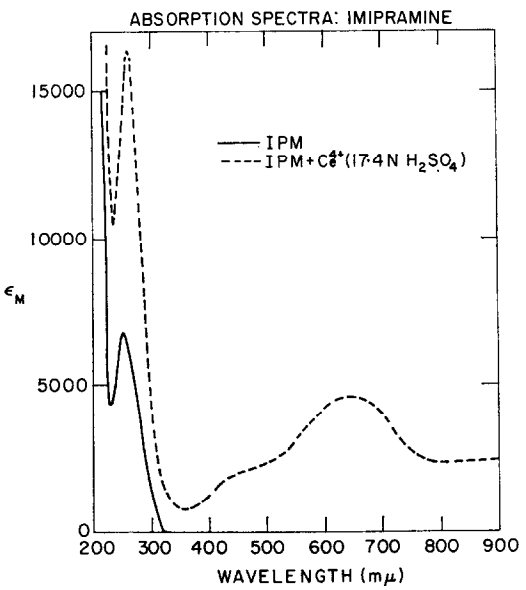

FIG. 1. Optical absorption spectra of imipramine hydrochloride (IPM) in water and of the blue oxidation product stabilized in sulfuric acid. The curves represent data combined from regions of different wavelengths obtained with cells of different optical path lengths.

salts or equimolar amounts of ceric ion (in 0·2 N H_2SO_4) produce immediately a deep blue color that fades in about 20 sec to a very pale green. In strong sulfuric acid ($\geqslant$ 10 N) the blue product remains stable for many hours. Its absorption spectrum has a broad peak at 640 mμ, and the u.v. absorption maximum of native imipramine at approximately 251 mμ shifts to 261 mμ (Fig. 1). On the other hand, H_2O_2 forms no chromophore from imipramine; but subsequent addition of horseradish peroxidase

slowly (seconds) produces the blue product, which then persists for 5 to 10 min, depending on the concentrations of the reactants. These reactions with univalent oxidizing systems suggest, by analogy with the phenothiazines,[11] that the blue product from imipramine may be a free radical.

Polarographic analysis of 0·0001 M imipramine in 0·2 N H_2SO_4 reveals a decomposition wave at a rotating platinum anode with an apparent $E_{1/2}$ of 0·82 V (Fig. 2)· The slope of the wave (Fig. 2) is intermediate between those expected for reversible univalent and bivalent oxidations.[15] However, irreversible processes are commonly encountered in the electrolysis of organic substances, and the slopes of their current-potential curves usually differ from those of reversible waves.[15]

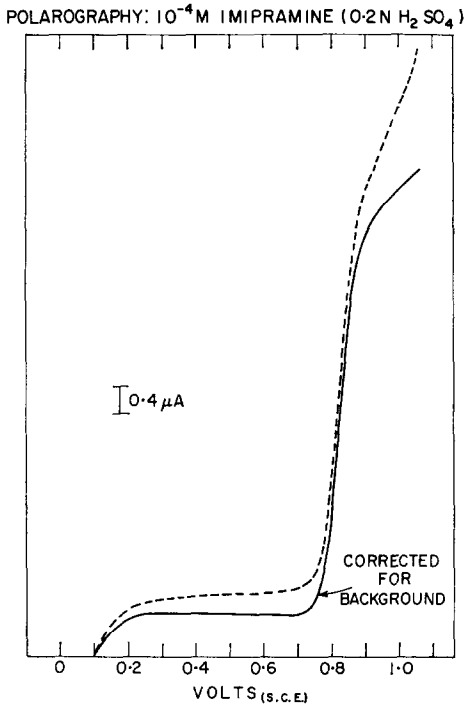

FIG. 2. Polarographic wave from imipramine (rotating platinum anode). A saturated calomel electrode (S.C.E.) was used for reference. The background was taken from 0·2 N H_2SO_4 alone.

Potentiometric titrations of imipramine with cerium confirm the irreversible nature of the oxidation. With the intermittent titration procedure each increment of ceric ion produces a dark blue color which fades in a few minutes, and simultaneously the solution potential jumps and then drifts slowly downward (Fig. 3). With 0·0005 M imipramine in 0·2 N H_2SO_4 the titration appears to be complete after about eight oxidizing equivalents have been added (Fig. 3). Potentiometric titration with cerium in 11·4 N H_2SO_4 reveals only a slight drifting of electrode potentials, and the titration appears complete after only two oxidizing equivalents (Fig. 4).

Electron paramagnetic resonance spectrometry confirms the presence of a free radical species after reaction with cerium. Because the blue reaction product is

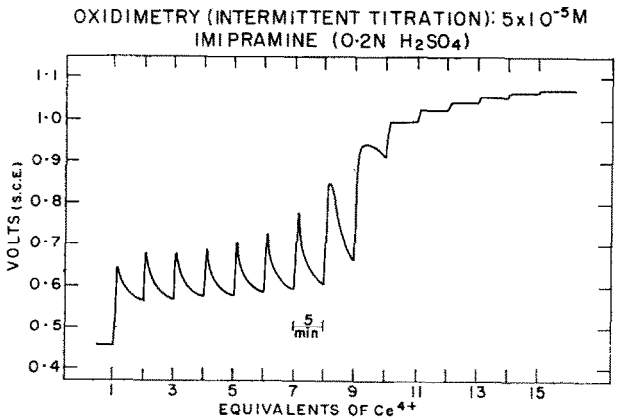

FIG. 3. Oxidimetric titration of imipramine. Each increment of approximately one equivalent of 0·01 N $H_2Ce(SO_4)_3$ was added in about 20 sec at the times indicated along the abscissa. The drift of solution potential between increments is seen to be largely completed after eight equivalents of oxidant and to terminate altogether thereafter.

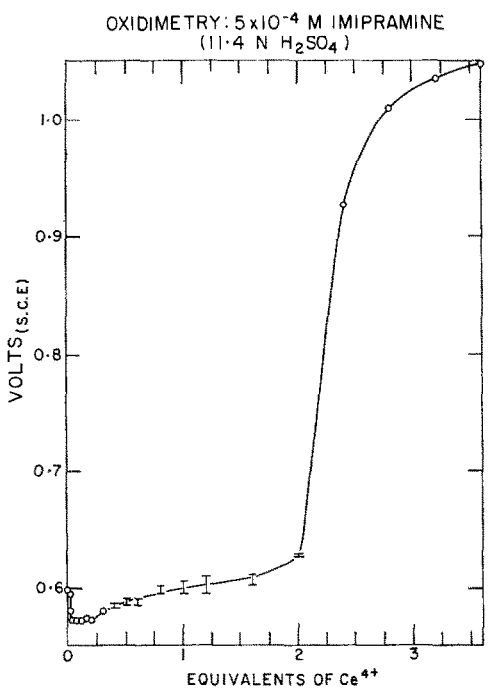

FIG. 4. Oxidimetric titration of imipramine in strong sulfuric acid. Increments of 0·02 NH_2 $Ce(SO_4)_3$ were added from a manually operated microburet. When the solution potential was unstable, the range of potential drift over a period of 2 min was recorded (vertical bars).

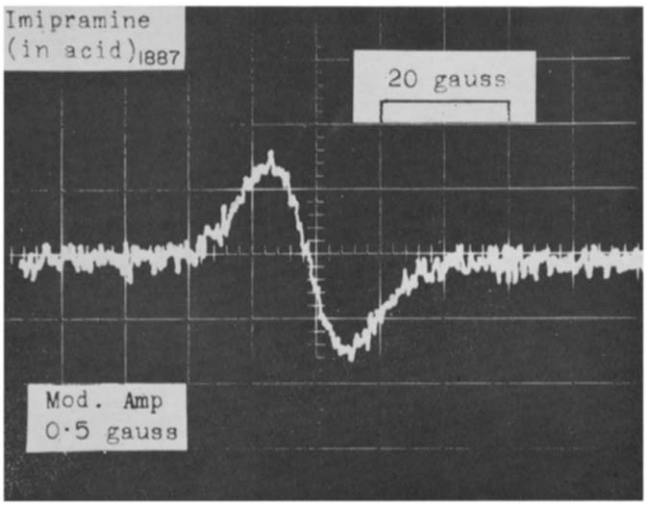

FIG. 5. EPR spectrum of free radicals from imipramine in mix-and-flow cell.[13] Reactants: 0·01 M imipramine. HCl in H_2O and 0·01 M $H_2Ce(SO_4)_3$ in 0·2 N H_2SO_4 mixing in 1:1 ratio at 20·5°. Flow conditions: spectrum scanned in 6 sec, beginning 2 sec after flow (at 6 ml/sec) is stopped. Resonance frequency: 9502·0 mc/s. Magnetic field increases from left to right, with intensity at midpoint of X-axis = 3392·0 gauss. Attenuation of microwave power: 3 db.

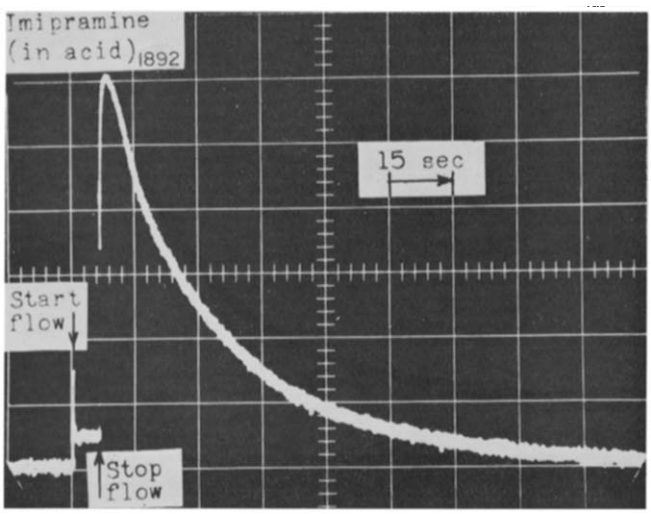

FIG. 6. EPR stop-flow curve. Ordinate: signal intensity; abscissa: time. Reactants as in Fig. 1. Flow conditions: at first arrow flow starts; steady state is maintained for 6 sec at flow rate of 6 ml/sec (corresponding to flow dead time ~4 msec between mixing and midpoint of microwave cavity); at second arrow flow stops and subsequent radical buildup and decay are recorded.

transitory, EPR was observed with a liquid flow system.[13] The EPR spectrum, recorded conventionally as the first derivative of microwave power absorbed versus magnetic field (Fig. 5), is a singlet with a total width of about 36 gauss and a *g*-value of 2·0026. The gaussian shape of the spectrum and its width are compatible with the presence of unresolved hyperfine structure; however, none was recorded even when EPR was observed with 100-kc modulation amplitudes as small as 0·25 gauss or when lower concentrations of reactants were used with reduced microwave power. Except for intensity, spectra were the same whether recorded during flow or after flow was stopped. Signal strength increased as flow rate decreased and reached a maximum a few seconds after flow ceased (Fig. 6), reflecting slow formation of the free radical. The subsequent decay of EPR intensity (Fig. 6) seemed to parallel the observed loss of blue color; but even as long as 25 min after reaction, a weak EPR singlet was obtained from the pale green solution, whose residual color and EPR could be quenched by addition of ascorbic acid. EPR of the blue free radical stabilized in sulfuric acid is the same as that seen in weak acid (Fig. 5).

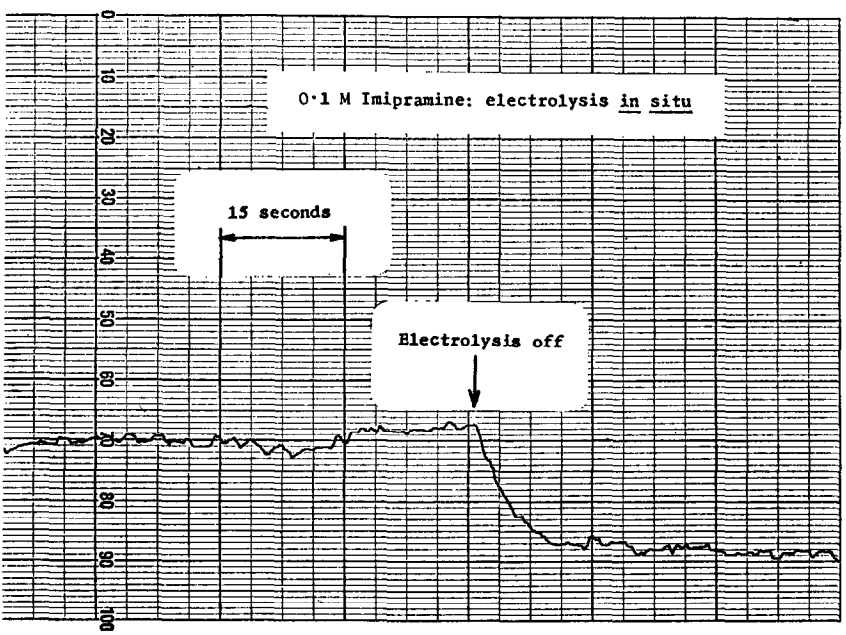

FIG. 7. Decay of impramine radical after electrolysis. Recorded trace represents intensity of free radical EPR signal at the anode as function of time (see text). First portion: equilibrium condition after 26 min of electrolysis at 3·0 V and 60 μA. At arrow: electrolysis abruptly discontinued.

Production of a free radical from imipramine can be confirmed further by generating it electrolytically. Electrolysis of 0·1 M imipramine hydrochloride at 1 to 3 V in a flat EPR sample cell allows EPR to be observed while anodic electrode products are generated *in situ*. An EPR signal can be recorded, and within a few minutes its intensity stabilizes, presumably as an equilibrium is established between free radical decay and the diffusion of unreacted imipramine to the electrode. The EPR spectrum is identical with that recorded after reaction with ceric ion (Fig. 5). Upon cessation of electrolysis, the EPR signal disappears in less than 15 sec (Fig. 7).

Electrolysis of imipramine in 11·4 N H_2SO_4 at 0·6 V produces a dark blue discoloration of the anode, which persists after electrolysis stops. EPR again demonstrates a single resonance peak, but the full spectral width from the radical on the anode surface is only about one half that seen in Fig. 5.

DISCUSSION

We have speculated that at least a part of the pharmacological actions shared by phenothiazine drugs may stem from their transformation to free radicals *in vivo*.[16] Since imipramine and chlorpromazine have common dimethylaminopropyl side chain substituents, similar distributions of the drugs within the body would be expected.[16] Thus the free radicals formed from both imipramine and phenothiazines could give rise to comparable pharmacological effects, especially the observed effects on oxidation and phosphorylation.[1, 3, 4]

The relatively slow formation of the free radical from imipramine (Fig. 6), the gaussian shape of its EPR spectrum (suggesting unresolved hyperfine structure; Fig. 5), the persistence of residual EPR signal for long times, and the irreversible reaction consuming many electron equivalents seen upon oxidimetry imply that there are complicating factors in free radical formation from this drug. More than one radical species may be present, other reactions may precede or accompany radical formation, or aggregation among radicals or between radicals and diamagnetic species may occur. However, the (final) production of metastable free radicals in weak acid is, in any case, analogous to semiquinone formation from phenothiazines in comparable circumstances.[11, 16] Furthermore, chromogenesis with manganous ion under conditions approaching neutrality also was observed with phenothiazines.[12] This finding implies that the free radicals that have been studied more readily in acid in these experiments with imipramine also form to some extent in a pH range of more direct biological interest.

REFERENCES

1. P. N. ABADOM, K. AHMED and P. G. SCHOLEFIELD, *Canad. J. Biochem.* **39**, 551 (1961).
2. G. BARTHOLINI, A. PLETSCHER and K. F. GEY, *Experientia (Basel)* **17**, 541 (1961).
3. S. LØVTRUP, *J. Neurochem.* **10**, 471 (1963).
4. M. J. CARVER, *Biochem. Pharmacol.* **12**, 19 (1963).
5. P. HELE, *Biochim. biophys. Acta* **76**, 647 (1963).
6. Y. KUROGOCHI, H. NISHIO, T. TAKADA, Y. TAKEBAYASHI and T. TSUJIOKA, *J. Nara med. Ass. (Japan)* **11**, 313 (1960).
7. J. M. RITCHIE and P. GREENGARD, *J. Pharmacol. exp. Ther.* **133**, 241 (1961).
8. F. HERR, J. STEWARD and M.-P. CHAREST, *Arch. int. Pharmacodyn.* **134**, 328 (1961).
9. I. P. LAPIN, *Psychopharmacologia (Berl.)* **3**, 413 (1962).
10. P. E. SIEGLER, *Med. Clin. N. Amer.* **48**, 483 (1964).
11. D. C. BORG and G. C. COTZIAS, *Proc. nat. Acad. Sci (Wash).* **48**, 623 (1962).
12. *Ibid.*, p. 617.
13. D. C. BORG, *Nature (Lond.)* **201**, 1087 (1964).
14. A. H. MAKI and D. H. GESKE, *J. Chem. Phys.* **30**, 1356 (1959).
15. P. DELAHAY, *Instrumental Analysis*, pp. 76, 81. Macmillan, New York (1957).
16. D. C. BORG and G. C. COTZIAS, *Proc. Nat. Acad. Sci. (Wash)* **48**, 643 (1962).