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NOVEL MEMBRANE LOCALIZED IRON CHELATORS AS INHIBITORS OF IRON-DEPENDENT LIPID PEROXIDATION

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Abstract—Attachment of various iron chelating moieties to hydrophobic steroids greatly enhanced their abilities to inhibit iron-dependent lipid peroxidation. Using whole rat brain homogenates, lipid peroxidation initiated by the addition of 200 μM Fe^{2+} was assessed by the formation of thiobarbituric acid reactive products (TBAR). Under these conditions, 50% inhibitory concentrations of Fe^{3+} chelators such as desferrioxamine or N^1,N^8-bis(2,3-dihydroxybenzoyl) spermidine hydrobromide (compound II) were around 170 and 50 μM respectively. Coupling desferrioxamine or compound II to a steroid at the D ring increased their potency in lipid peroxidation assays by 5- to 10-fold. Evidence that inhibition of lipid peroxidation by the steroid–chelator adducts was due to iron chelation was suggested by the fact that methylation of the catechol oxygens of compound II, which are essential for chelation, completely eliminated activity of the steroid adduct. A series of 21-aminosteroids which complex Fe^{2+} iron and potently inhibit iron-dependent lipid peroxidation has also been synthesized. Coupling Fe^{2+} chelators to hydrophobic steroids increased their inhibitory potencies by as much as 10- to 100-fold. Some steroid-based Fe^{2+} chelators stimulated lipid peroxidation at low concentrations in the presence of Fe^{3+}. The degree of stimulation was related to the affinity of a compound for Fe^{2+} with the stronger chelators causing greater stimulation. The most potent inhibitors of lipid peroxidation in the 21-aminosteroid series were found to be those compounds forming the weakest Fe^{2+} complexes. The findings suggest that it is iron at or near the membrane that is responsible for the catalysis of lipid peroxidation. The compounds described should provide useful tools for studies of the involvement of iron in the lipid peroxidation process.

The role of iron in lipid peroxidation, although perhaps not clearly understood, is nevertheless well recognized. Both Fe^{2+} and Fe^{3+}, or their respective chelates, can precipitate oxygen radical formation and may either initiate, or participate in, lipid peroxidation reactions under a variety of diverse circumstances. Although much attention has been focused on the iron catalyzed formation of hydroxyl radical ($OH^{\cdot}$) as the important step for initiation of lipid peroxidation [1–3], iron catalyzed decomposition of lipid hydroperoxides (LOOH) may be a primary driving force for lipid peroxidation [4, 5]. Furthermore, the role of $OH^{\cdot}$ in the initiation process has been called into question by the work of a number of investigators [6–11]. In many cases, formation of the perferryl or ferryl iron species [8, 9] or the absolute ratio of Fe^{2+} to Fe^{3+} has been hypothesized as being important for initiation of lipid peroxidation [10, 11]. In either case, a central role for iron in lipid peroxidation cannot be questioned.

Girotti and Thomas [2] have hypothesized that it is oxygen radicals generated at or on the membrane by iron that are responsible for iron initiated lipid peroxidation. Several investigators have indeed suggested that iron which initiates or participates in lipid peroxidation may actually be bound or solubilized by membrane lipids [12–15]. With this in mind, we have tested whether or not the potencies of iron chelators as inhibitors of lipid peroxidation could be enhanced by attaching them to lipophilic steroids, thereby targeting them to the membrane. In the present study, a novel series of steroid-based iron chelators was examined for their ability to inhibit iron-dependent lipid peroxidation. The 21-amino steroids described in this report represent a new class of pharmacological agent and have shown excellent activity in *in vivo* models of central nervous system trauma and ischemia [16–18 ‖].

MATERIALS AND METHODS

Whole rat brain homogenates were freshly prepared in Krebs buffer containing 15 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4, 10 mM glucose, 140 mM $NaCl_2$, 3.6 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM KH_2PO_4 and 0.7 mM $MgCl_2$. Male rats (150–175 g) were decapitated, and the brains (minus cerebellum) were

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rapidly removed and homogenized in 10 vol. of ice-cold Krebs buffer using a glass Dounce homogenizer. Homogenates were used immediately in lipid peroxidation assays where indicated. In some cases purified rat brain synaptosomes were prepared as described [19] with some modification [10] and were used immediately. Lipid peroxidation was assessed in 100-μl incubations at 37° by the formation of thiobarbituric acid reactive products (TBAR). Incubations containing brain homogenate were in Krebs buffer and those containing synaptosomes were in 0.9% NaCl in which the pH was carefully adjusted to 7.0 immediately prior to use [10]. Peroxidation reactions were initiated by addition of either Fe^{2+} or Fe^{3+} as indicated. Reactions were stopped by the addition of 500 μl of ice-cold 0.8 N HCl containing 12.5% trichloroacetic acid. TBAR formation was then determined as described elsewhere following the addition of 50 μM desferrioxamine prior to boiling [10]. Solutions of Fe^{2+} or Fe^{3+} were prepared fresh in argon purged H_2O as described [10] and were used immediately. Fe^{3+} prepared in this manner has been shown to contain approximately 2% Fe^{2+} [10]. Results are the means of triplicate determinations from representative experiments with a variance of less than 6%, N = 3 or 4.

The synthesis of U74915 (see Fig. 1B) was as follows: A mixture containing 1.49 g of 3-methoxyesterone, 2.0 g of desferrioxamine mesylate (DESFERAL, Ciba) and 47 g of 4A molecular sieves in 100 ml of tetrahydrofuran and 200 ml of methanol was adjusted to pH 6 with glacial acetic acid and then treated with 188 mg of sodium cyanoborohydride. The resulting heterogeneous mixture was heated at reflux under nitrogen for 90 hr. The reaction mixture was cooled to room temperature, filtered through Celite and evaporated, and the residue was purified by chromatography on silica gel (80/15/5 chloroform/methanol/concentrated ammonium hydroxide). The purified product, U74915 (100 mg), was characterized by mass spectroscopy and TLC: $(M + H)^+$ observed at m/z 829. 5431; calcd. for $C_{44}H_{73}N_6O_9$, 829.5439; other ions at m/z 352, 243, 173, 159, and 147; R_f 0.56 (silica gel plates, 80/15/5 chloroform/methanol/concentrated ammonium hydroxide).

The synthesis of U75014E was as follows: To a solution of N^1,N^8-bis(2,3-dimethoxybenzoyl) spermidine [20] (0.36 g, 0.76 mmol) in 5 ml of acetonitrile was added Na_2CO_3 (160 mg, 1.5 mmol) and 21-bromo-17α-hydroxy-4,9-(11)-pregnadiene-3,20-dione (0.28 g, 0.68 mmol). The resulting suspension was refluxed for 2 hr under nitrogen. After cooling, the suspension was diluted with 10 ml CH_2Cl_2 and filtered to remove the Na_2CO_3. After evaporation of the solvent, the resulting solid was chromatographed on silica gel eluting with ethanol-$CHCl_3$/NH_4OH (5/95/0.1) to yield 200 mg (74%) of the desired product as a white solid which was characterized as described above. $(M + H)^+$ observed at m/z 799.4407; calcd. for $C_{46}H_{61}N_3O_9$, 799.4410.

U75014E was methylated to U75013E as follows: To a solution of U75014E (0.21 g, 0.26 mmol) in 10 ml CH_2Cl_2 at −78° under N_2 was added 3.9 ml of a 1 M BBr_3 solution in CH_2Cl_2. The resulting suspension was allowed to warm to room temperature with continued stirring. After 45 min, the flask was cooled to 0° and 5 ml of ice water was added with vigorous stirring. The resulting solid was collected by filtration under N_2. The crude product was purified by reverse phase chromatography on an RP-8 column eluting with 35% CH_3CN/0.1% CF_3CO_2H/0.075% triethylamine. The cleanest fractions were pooled, concentrated by evaporation to a small volume, and product extracted into ethyl acetate. After washing the triethylamine with dilute CF_3CO_2H, drying with Na_2SO_4 and solvent removal, 70 mg of the trifluoroacetate salt of the desired compound was obtained as a pale yellow solid. The final product was characterized as described above. $(M + H)^+$ observed at m/z 743.3782; calcd. for $C_{42}H_{53}N_3O_9$, 743.3784.

The synthesis of U76556, U75412A. U77372E, U78000E and U74500A was as described in U.S. patent application No. 4236 and European patent application No. 4236.P. Structures were determined by NMR and mass spectroscopy. Details concerning the synthesis of any of the compounds described in this report will be provided by the authors on request.

Ultraviolet and visible spectral analyses of compounds were conducted using a Tracor Northern 6500 diode array spectrophotometer.

All materials were obtained commercially and were the highest grade available.

RESULTS

Desferrioxamine (Fig. 1B), a potent chelator of Fe^{3+}, is commonly used to inhibit lipid peroxidation reactions involving iron. The addition of 200 μM Fe^{2+} to rat brain homogenates in Krebs buffer resulted in formation of TBAR that could be inhibited by desferrioxamine in a dose-dependent manner (Fig. 1A). Compound II (Fig. 1B) is another potent iron chelator with selectivity for Fe^{3+} which was originally isolated from *Micrococcus denitrificans* [20, 21]. Compound II has an Fe^{3+} complex formation constant of 10^{40}, while that for desferrioxamine is around 10^{29} [22]. In the lipid peroxidation assay using whole brain homogenate, compound II inhibited TBAR formation and was nearly ten times more potent than desferrioxamine (Fig. 1A). Coupling desferrioxamine or compound II to a steroid moiety at C-17 (U74915) or C-21 (U75014E) positions, respectively (Fig. 1B), increased their potency in the lipid peroxidation assay by at least a factor of 10 (Fig. 1A). Iron chelation by the steroid–chelator adducts was absolutely essential for activity since methylation of the catechol hydroxyl groups of compound II, which are necessary for metal complex formation [22], yielded the inactive steroid adduct, U75013.

Just as desferrioxamine and compound II preferentially chelate Fe^{3+}, certain pyridine based compounds are known to form stable complexes with Fe^{2+}. Tripyridinyltriazine (TPT; Fig. 2B), for example, readily forms a stable complex with Fe^{2+} and has been used in assays for Fe^{2+} [23, 24]. TPT did not inhibit lipid peroxidation in whole brain homogenates with Fe^{2+} (Fig. 2A), but rather, marked stimulation was observed at the concentrations tested. However, a monopiperazinyl-

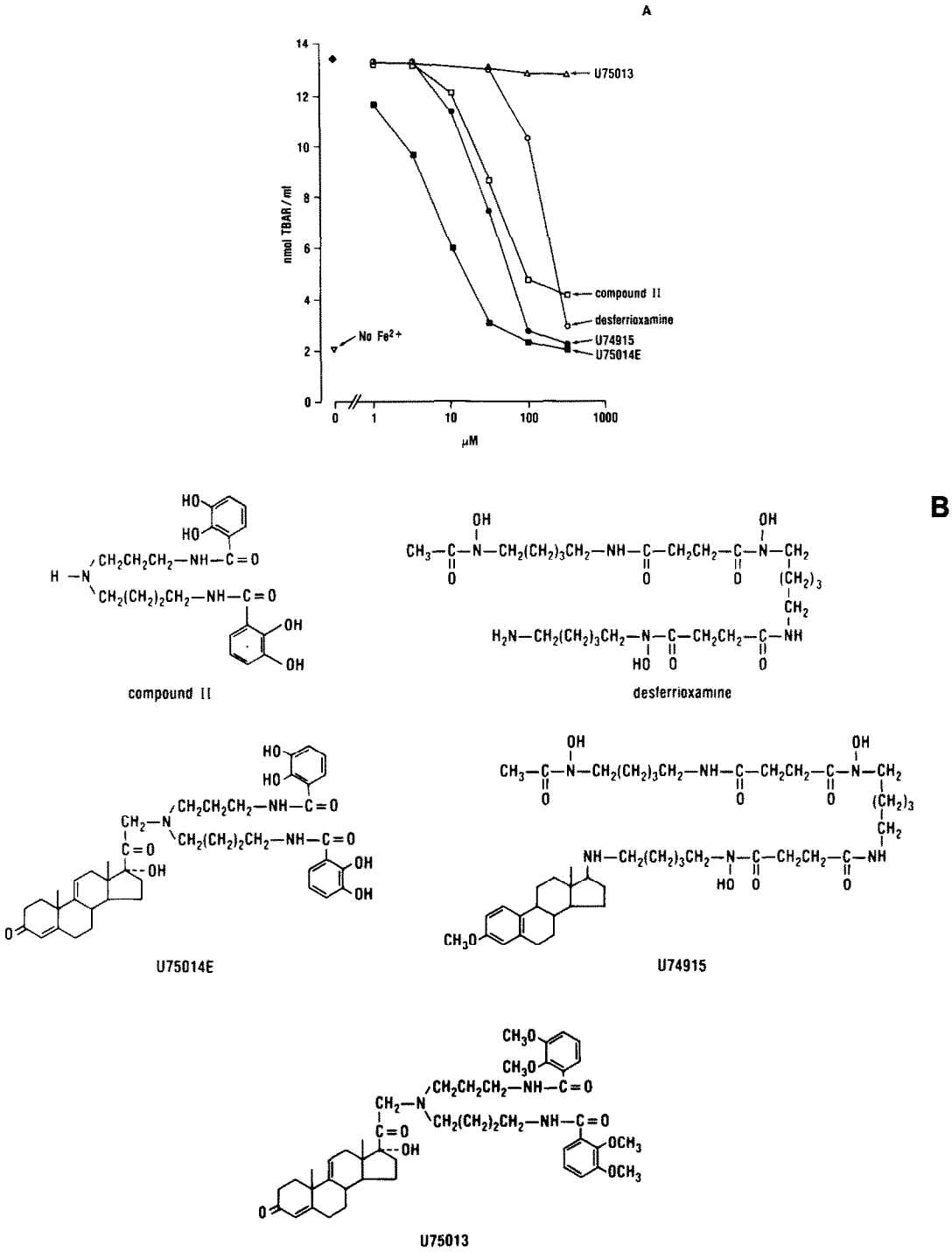

Fig. 1. Inhibition of lipid peroxidation in brain homogenate by Fe^{3+} chelators. (A) Rat brain homogenates (10 μl) were incubated in 100 μl Krebs buffer at 37° for 20 min in either the absence of 200 μM Fe^{2+} (∇) or its presence (◆). Reactions were initiated by the addition of Fe^{2+}, and TBAR formation was determined as described in Materials and Methods. Some incubations with Fe^{2+} also contained the compounds in (B) at the concentrations indicated. Compounds were dissolved in ethanol with the exception of desferrioxamine, which was soluble in H_2O. Controls with and without Fe^{2+} contained 10% ethanol as a final concentration corresponding to that contributed by inclusion of test compounds in the assay. Ethanol did not affect the formation of TBAR.

dipyridinyl-triazine analog of TPT attached to a steroid (U77372E, Fig. 2B) did inhibit iron-dependent TBAR formation in a dose-dependent manner (Fig. 2A). U77372E also stimulated lipid peroxidation somewhat at low concentrations although not to the degree seen with TPT itself. Another 21-aminosteroid, U78000E (Fig. 2B), which contains a monopiperazinyl-dipyridinyl-pyrimidine, was a slightly less potent inhibitor of lipid peroxidation while being slightly more stimulatory. The most potent inhibitors of lipid peroxidation in the 21-aminosteroid chemical series were U75412A and U74500A (Fig. 2, A and B). Both U75412A and U74500A contain amino-substituted-pyridinyl-piperazines possessing the N—C—C—N fragment common to Fe^{2+} chelators such as TPT. Neither

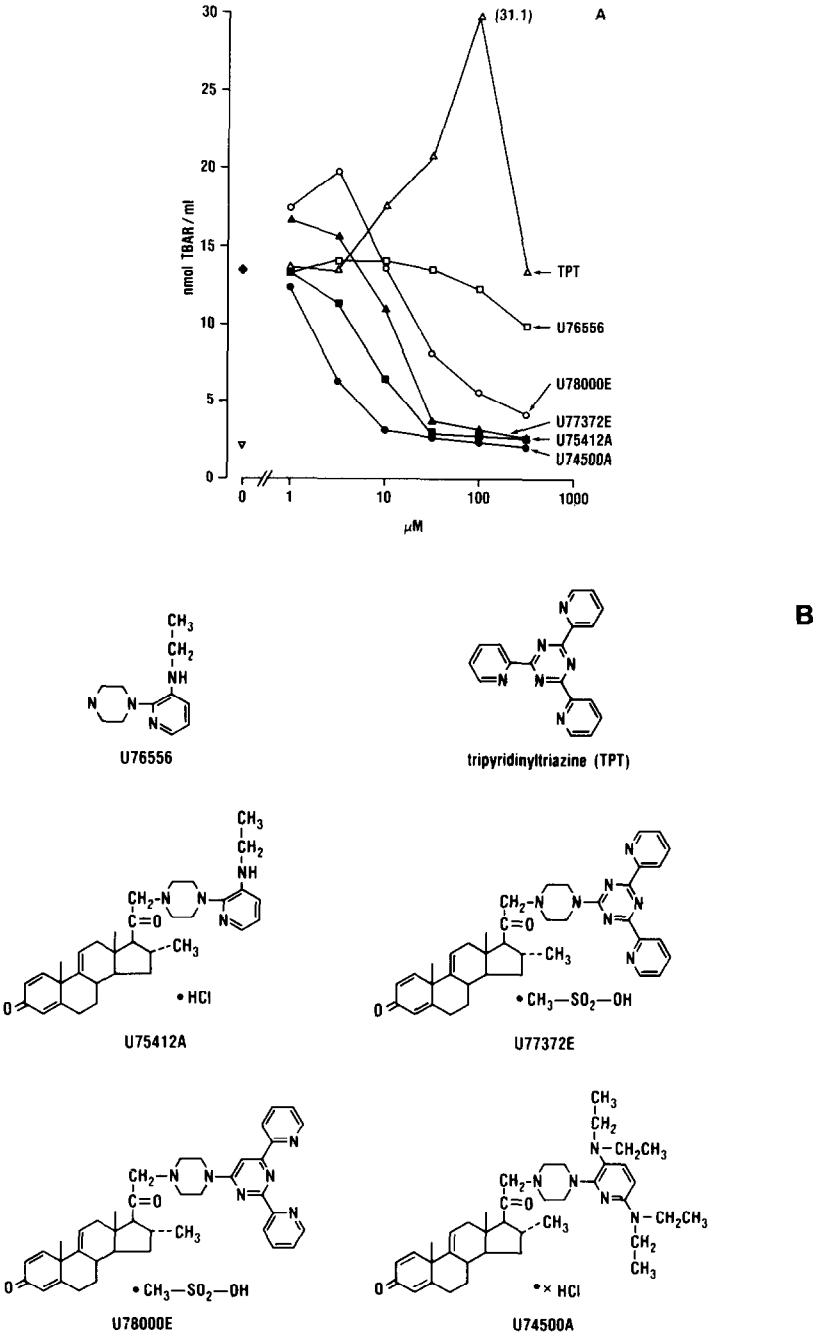

Fig. 2. Inhibition of lipid peroxidation in brain homogenate by 21-amino steroids and Fe^{2+} chelators. (A) Rat brain homogenates (10 μl) were incubated in 100 μl Krebs buffer at 37° for 20 min in either the absence of 200 μM Fe^{2+} (▽) or its presence (◆). Assay conditions were the same as in Fig. 1A. Some incubations with Fe^{2+} also contained the compounds in (B) at the concentrations indicated. All compounds were prepared in H_2O.

U74500A nor U75412A stimulated lipid peroxidation under the assay conditions in Fig. 2, and both were excellent inhibitors. The 21-amino fragments of U75412A (U76556; Fig. 2, A and B) or U74500A (not shown) only weakly inhibited lipid peroxidation when not attached to a steroid. As suggested by the data in Fig. 1A for U75013, the steroid fragment itself was only a very weak inhibitor of lipid peroxidation, reducing TBAR formation by less than 7% at a concentration of 1 mM [16].

The ability of the 21-aminosteroids to inhibit lipid peroxidation in the brain homogenate system was related to their affinity for Fe^{2+}. The 21-aminosteroids having the lowest affinity for Fe^{2+} (presumably forming the weakest Fe^{2+} complex) were the more potent inhibitors in the series. Figure 3 shows examples of iron-dependent UV spectral changes with U74500A and U77372E following addition of either 4 or 20 μM Fe^{2+}. While U77372E displayed marked spectral shifts dependent upon the concentration of added Fe^{2+}, U74500A spectral changes were much less under the same conditions. The iron-dependent spectral changes for each compound occurred largely in the 260–280 nm region where aromatic amines characteristically absorb. These findings suggest that iron complex formation occurred at the amino portion of the molecule.

The stronger iron chelating compounds U77372E and U78000E also displayed spectral changes with Fe^{2+} in the visible range (Fig. 4) similar to those observed for TPT [23, 24]. No visible spectral shifts were observed with U74500A (Fig. 4) or U75412A. A preference for Fe^{2+} was suggested by the fact that none of the compounds displayed UV or visible spectral shifts in the presence of Fe^{3+}, suggesting that they had very little affinity for reduced iron (not shown). Consistent with its greater spectral shift in the presence of Fe^{2+}, U78000E apparently formed a thermodynamically more stable complex with Fe^{2+} than did U77372E (Fig. 5). While the U77372E–ferrous complex could be readily dissociated by the addition of small amounts of EDTA, the U78000E–ferrous complex was relatively stable over time in the presence of a large excess of EDTA. After several hours in the presence of EDTA, however, dissociation of the U78000E–Fe^{2+} complex could be detected (not shown). Despite the apparent differences in their iron complex stability, both U78000E and U77372E displayed a 3:1 drug:ligand stoichiometry (Fig. 5).

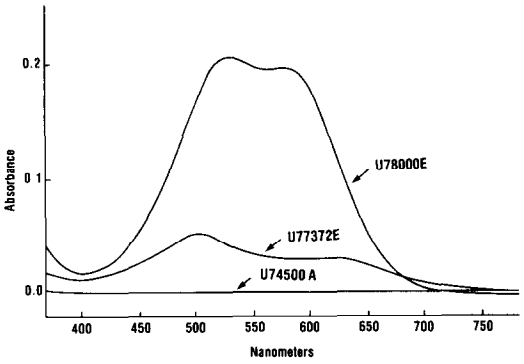

Fig. 4. Difference visible spectra of U78000E, U77372E and U74500A in the presence of Fe^{2+}. Fifty micromolar solutions of U78000E, U77372E or U74500A in H_2O were scanned 4 sec following the addition of 20 μM Fe^{2+}. Represented are difference spectra of compound plus Fe^{2+} minus spectra of compound alone.

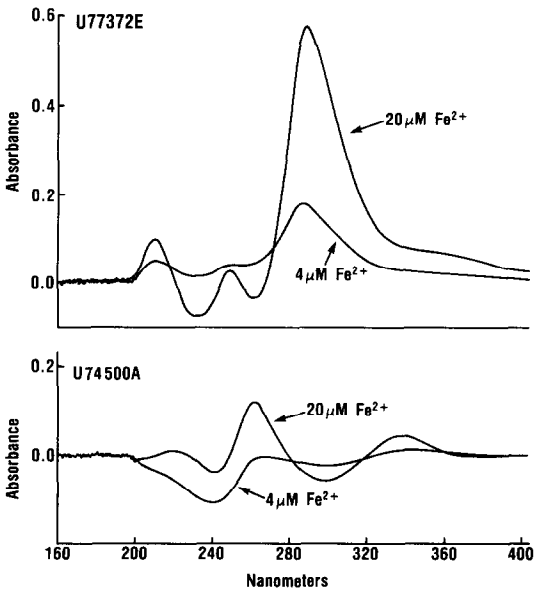

Fig. 3. Difference UV spectra of U77372E and U74500A in the presence of Fe^{2+}. Fifty micromolar solutions of U77372E or U74500A in ethanol were scanned 4 sec following the addition of either 4 or 20 μM Fe^{2+} as indicated. Represented are the difference spectra of compound plus Fe^{2+} minus spectra of compound alone.

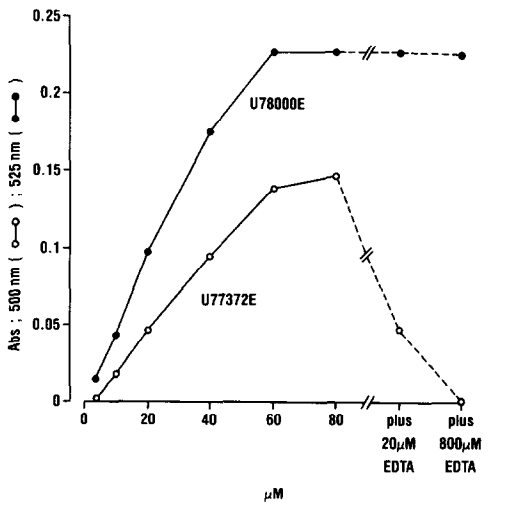

Fig. 5. Iron binding of U77372E and U78000E. The concentrations of U77372E (absorbance read at 500 nm, ○—○) or U78000E (absorbance read at 525 nm, ●—●) indicated were added in a cumulative manner in 20 μM increments to a 20 μM solution of Fe^{2+} in H_2O and the absorbance was read. Following absorption readings with 80 μM compound, 20 μM EDTA followed by 800 μM EDTA (---) were added to the curvettes containing Fe^{2+} and compound as indicated. Absorbance readings after EDTA were taken 1 min after its addition.

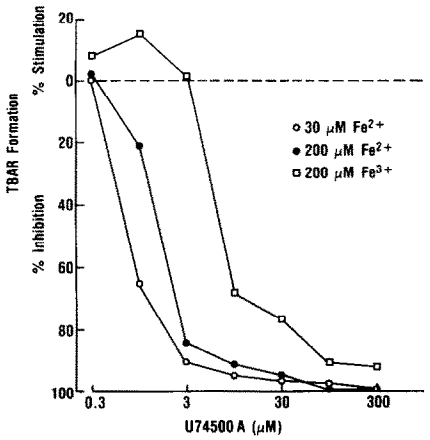

Fig. 6. Inhibition of lipid peroxidation by U74500A in saline. Purified rat brain synaptosomes (10 μl) were incubated at 37° in 100 μl of 0.9% NaCl, pH 7.0, for either 20 min (○, □) or 60 min (●), and the formation of TBAR was determined. Reactions contained the concentration of U74500A indicated and were initiated by the addition of either 30 μM Fe^{2+} (○——○), 200 μM Fe^{2+} (●——●) or 200 μM Fe^{3+} (□——□). Total TBAR formed in the absence of U74500A were 10.5 nmol/ml (30 μM Fe^{2+}), 25.1 nmol/ml (200 μM Fe^{2+}), and 19.7 nmol/ml (200 μM Fe^{3+}).

Using purified brain synaptosomes in unbuffered saline, the inhibitory actions of U74500A and U78000E were examined further. The inhibitory effects of the 21-aminosteroids on lipid peroxidation were apparently dependent upon both the concentration of iron and its oxidation state. Although U74500A inhibited TBAR formation following the addition of 200 μM Fe^{2+} to synaptosomes in saline, the compound was more potent in the presence of 30 μM Fe^{2+} (Fig. 6). These results would be anticipated for a compound with an affinity for Fe^{2+}. U74500A was less potent in assays containing Fe^{3+} (Fig. 6). Also, with Fe^{3+}, some stimulation of TBAR formation could be observed at the lower concentrations of drug.

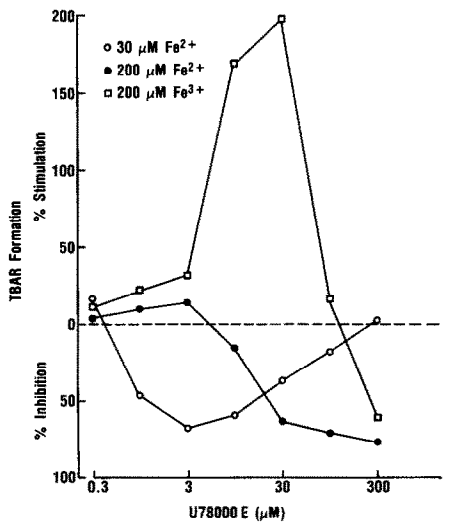

Fig. 7. Inhibition of lipid peroxidation by U78000E in saline. Conditions were identical to those in Fig. 6 except that incubations contained U78000E at the concentrations indicated.

The effects of the strong iron chelating amino steroid U78000E were also examined under conditions identical to those described for U74500A. While U78000E also did not stimulate lipid peroxidation significantly in unbuffered saline with Fe^{2+} (Fig. 7), marked stimulation was observed with Fe^{3+}. In the case of Fe^{2+}, U78000E inhibited more potently in the presence of 30 μM Fe^{2+} than with 200 μM Fe^{2+}. However, in the presence of 30 μM Fe^{2+}, as the concentration of U78000E was increased, its inhibitory capacity was lost. It is possible that loss of inhibitory activity of U78000E at higher concentrations with 30 μM Fe^{2+} was perhaps due to decompartmentalization of endogenous iron by the strong chelator. Evidence for this was given by the observation that, at high concentrations, U78000E stimulated lipid peroxidation even in the absence of added iron, an effect that was enhanced by the presence of low concentrations of ascorbate (data not shown).

Finally, the effects of premixing the 21-aminosteroids with Fe^{2+} on their abilities to inhibit iron-dependent lipid peroxidation were examined (Fig. 8). Just as with other chelators such as desfer-

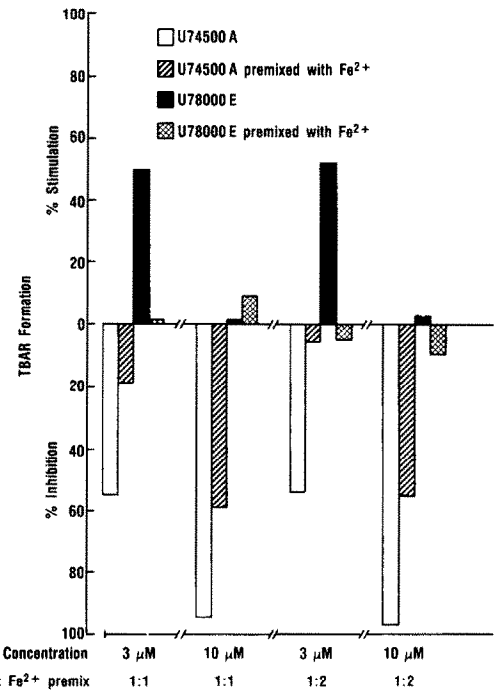

Fig. 8. Inhibition of lipid peroxidation by U74500A or U78000E premixed with Fe^{2+}. Rat brain homogenate (10 μl) was incubated for 20 min at 37° in Krebs buffer (100 μl) and TBAR formation was determined. Reactions were initiated by the addition of 200 μM Fe^{2+}. Control TBAR formed in the absence of drug was 13.9 nmol/ml. Some incubations also contained either U74500A (□, ▨) or U78000E (■, ▩) as indicated. In some instances, drug was first premixed with Fe^{2+} in either a 1:1 or 1:2 (drug:Fe^{2+}) ratio as indicated prior to addition into the incubation. The additional Fe^{2+} added (up to 20 μM) along with the drug had no effect on the amount of TBAR formed in the presence of 200 μM Fe^{2+}.

rioxamine, prior exposure to Fe^{2+} reduced the activity of the 21-aminosteroids in the lipid peroxidation assay. In the case of U78000E, both stimulatory and inhibitory actions were blunted by prior exposure to Fe^{2+}.

DISCUSSION

The studies described report our preliminary findings with a novel series of lipid peroxidation inhibitors. These compounds were developed specifically to target iron chelators to biological membranes by coupling the chelating moiety to a hydrophobic steroid. It is well known that steroids are highly lipophilic and readily intercalate or partition into cell membranes and phospholipid environments [25, 26]. Based upon their retention factors (k') obtained from a C-18/water HPLC system [27], the steroid–chelator adducts described in this report were highly lipophilic; in most cases they were more lipophilic than the steroids themselves (Braughler, unpublished observations). The results demonstrate that, by coupling iron chelators to a hydrophobic steroid moiety, their abilities to inhibit iron-dependent lipid peroxidation can be greatly enhanced.

By targeting the chelating moiety to the membrane where iron participates in lipid peroxidation, chelation is not wasted on iron in the aqueous environment. On a molecular scale, the majority of iron added to a lipid peroxidation assay may be far removed from hydrophobic regions of the membrane where iron catalyzed lipid peroxidation reactions occur. The idea that it is iron bound at or near the membrane which is responsible for the catalysis of lipid peroxidation has been suggested by the work of a number of investigators [2, 12–15]. The present results suggest that this idea is certainly worthy of consideration. The fact that coupling a chelator to a hydrophobic steroid increases its potency as an inhibitor of iron-dependent lipid peroxidation suggests that only a small fraction of the total iron available in an assay actually participates in lipid peroxidation. For example, in order for desferrioxamine or other chelators to inhibit lipid peroxidation completely, their concentration must be in a molar excess of the total iron added. Steroid–chelator adducts, on the other hand, effectively inhibited lipid peroxidation at concentrations far below the concentration of endogenously added iron present in the assay. The steroid itself apparently contributes little antioxidant activity. We have prepared over 200 compounds in the 21-aminosteroid chemical series, and activity is critically dependent upon the structure of the amine fragment.*

Some striking differences were observed in the behaviors in lipid peroxidation assays of Fe^{2+} chelators and Fe^{3+} chelators. While Fe^{3+} chelators and their steroid–chelator adducts inhibited lipid peroxidation in Krebs buffer, strong Fe^{2+} chelators or their steroid adducts both stimulated as well as inhibited lipid peroxidation in Krebs buffer. Fe^{2+} auto-oxidation in Krebs buffer probably results from the complexing of Fe^{3+} by phosphate which rapidly shifts the equilibrium between Fe^{2+} and Fe^{3+} in favor of Fe^{3+}. Fe^{2+} auto-oxidation is indeed quite rapid (within several seconds) in Krebs buffer as evidenced by the rapid uptake of oxygen and loss of Fe^{2+} in Krebs (or phosphate) buffer following addition of Fe^{2+} ([5] and Braughler, unpublished observations). Lipid peroxidation will proceed with either Fe^{3+} or Fe^{2+} provided that a mechanism exists to facilitate the interconversion of iron between its redox states [10, 11]. A chelating agent may serve as such a catalyst as either Fe^{2+} or Fe^{3+} becomes bound to the chelator, thereby shifting the equilibrium in the direction of the chelated form (see below).

In the case of Fe^{3+} chelators or their steroid adducts, in Krebs buffer only inhibition of lipid peroxidation should be observed. This would be expected since Fe^{3+} chelators, along with phosphate present in the buffer, should act to maintain (or trap) iron in the Fe^{3+} form. On the other hand, strong Fe^{2+} chelators may stimulate lipid peroxidation in the presence of Krebs by favoring conversion of Fe^{3+} to Fe^{2+}. The conversion of Fe^{3+} to Fe^{2+} may result from either direct reduction of Fe^{3+} by the compound itself, a shift in the equilibrium between Fe^{3+} and Fe^{2+} caused by Fe^{2+} chelation, or the enhanced oxidation potential of Fe^{3+} in the presence of strong Fe^{2+} chelators. Further studies will be required to delineate the precise mechanisms involved in the stimulation of lipid peroxidation by these compounds. In unpublished studies, however, both TPT and U78000E have been shown to cause the slow conversion of Fe^{3+} to Fe^{2+} when added to solutions containing Fe^{3+} in the absence of lipid. The rate of conversion of Fe^{3+} to Fe^{2+} by TPT or U78000E was enhanced markedly by the presence of a readily oxidizable lipid substrate (Braughler, unpublished observations). Fe^{2+} chelators should be more stimulatory to lipid peroxidation with Fe^{3+} under conditions where less competition for Fe^{3+} exists (by phosphate for example), i.e. in unbuffered saline. The data are in excellent agreement with this idea since U78000E was more stimulatory in saline with Fe^{3+} than in Krebs. On the other hand, neither U78000E nor U74500A, both of which possess Fe^{2+} binding characteristics, stimulated lipid peroxidation in saline with Fe^{2+}.

The findings that either Fe^{2+} or Fe^{3+} chelators or their steroid–adducts could inhibit lipid peroxidation under the appropriate conditions, along with the finding that targeting of these compounds to the membrane increased their potency as inhibitors, allow tentative conclusions to be drawn concerning the participation of iron in lipid peroxidation. These observations lend support to the idea that it is iron either at or in the membrane which is responsible for the initiation and propagation of lipid peroxidation reactions. The results further suggest that, *in vitro*, only a fraction of available iron added to incubations actually participates in lipid peroxidation. The compounds described may be useful tools in the study of the mechanisms involved in iron-dependent lipid peroxidation.

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