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DIFFERENTIAL EFFECTS OF *IN VITRO* PEROXIDATION ON PERIPHERAL- AND CENTRAL-TYPE BENZODIAZEPINE RECEPTORS

PROTECTION BY DIVERSE ANTIOXIDANTS

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Abstract—The influence of various concentrations of ferrous iron and ascorbate on *in vitro* peroxidation and drug binding of diverse membrane preparations (cerebral cortex and liver) was studied. Peroxidation was not simply dose-related to ascorbate and ferrous iron, but a complex relationship between iron and ascorbate when added in association was established. Under our conditions 0.01 mM Fe^{2+} and 0.5 mM ascorbate was the most peroxidative combination for cerebral and liver membranes. Under the same conditions, cerebral membranes were more peroxidated than liver membranes. Considering the consequences of drug binding, peripheral-type benzodiazepine receptors (PBRs) of liver were more affected by peroxidative events than central-type benzodiazepine receptors (CBRs) of the cerebral cortex. The degree of binding disturbance was generally inversely correlated to the degree of peroxidation and this was more significant for liver PBRs than for cerebral CBRs. The liver membrane model was retained for testing *in vitro* protection by diverse putative antioxidants. Under our conditions desferrioxamine, ethylene diamine tetra acetate (EDTA), trolox, and rutin were good protective antioxidants, whereas phenyl-butyl-nitrone (PBN) and tocopherol were not effective.

Key words: peroxidation; central-type benzodiazepine receptor; peripheral-type benzodiazepine receptor; oxygen toxicity; antioxidant; rat

Biological membranes are dedicated to cellular compartmentation and communication; they constitute a functional connection between cells and between organelles. This connective role is supported by hormone/neurotransmitter receptors and ion channels. These macromolecules are glycoproteins and are embedded in a phospholipidic bilayer; their functional role is not solely due to their glycoproteic moiety but to the lipid microenvironment as well. In this way, it is obvious that any structural disturbance to membrane components (protein or lipid) may have important consequences on receptor functioning.

Oxygen radicals are physiological species of oxygen metabolism but can sometimes occur at exaggerated levels during pathophysiological processes. In this case, structural as well as functional disturbances consecutive to oxidative damage are produced.

Hyperoxia is one of those pathological processes that enhance oxygen radical fluxes. We have recently explored the modifications of binding capacities of cerebral and liver membranes prepared from hyperbaric oxygen-exposed rats [1]. In this study, the binding capacity of cerebral membranes for [^{3}H]-flunitrazepam (corresponding to CBR†) was reduced after hyperoxic exposure and the binding capacity of cerebral and liver membranes for [^{3}H]-Ro 5-4864 (corresponding to PBR) was unaffected. However, it was difficult to account for the actual mechanisms of such modifications. Reduced density of receptors might be a consequence of "down-regulative" events, but may also be due to direct degradative processes on receptors themselves or to peroxidation of their lipid environment. On the other hand, some questions about the different susceptibility of CBRs and PBRs to hyperoxia are raised. Is the diminished density of the CBR consecutive to its intrinsic sensitivity or to differential susceptibility of membrane components? Is the differential sensitivity of PBRs and CBRs not essentially due to their particular location in the cell and to a more protective environment of one with regard to the other?

The present work was conducted *in vitro* so that the differential sensitivity of peripheral- and central-type benzodiazepines might be understood and influence of diverse cofactors of the peroxidative process explained. Because lipid peroxidation is not necessarily the sole cause of structural or functional disturbances in membrane receptors due to oxidative damage, it was important to study both the peroxidative process and receptor-binding consequences in the same experiment. Thus, it was interesting to study the relation between the peroxidation of membranes and the binding capacity of receptors, in connection with diverse levels of "biological partners" such as ferrous iron and ascorbate, separately or in association. In the second part of the study, this *in vitro* model was used to test putative "antioxidants" for their ability to protect receptor-binding capacity.

MATERIALS AND METHODS

Preparation of tissue homogenates

Concerning cerebral homogenates, a crude synaptosome-rich fraction (P_2) was obtained by initial homogenizing in 20 volumes of 0.32 M sucrose and subsequent centrifugations, as described by Squires and Braestrup [2].

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† Abbreviations: CBR, central-type benzodiazepine receptor; PBR, peripheral-type benzodiazepine receptor; MDA, malonedialdehyde; EDTA, ethylene diamine tetra acetate; PBN, phenyl butyl nitrone.

Crude liver homogenates were obtained by homogenizing with UltraTurrax and subsequent centrifuging. All tissue homogenates were washed 3 times in 50 mM Tris-HCl buffer pH 7.4 and were subsequently stored at –80°C.

Induction of lipid peroxidation

Cerebral and liver membranes were thawed and diluted in Tris-HCl to obtain a protein concentration of 0.5–2 mg/mL. 200 µL fractions were equilibrated at 37°C in glass tubes and then incubated with or without diverse ferrous sulphate concentrations (0/0.01/0.1/1 mM), in association (or not) with various ascorbate concentrations (0/0.1/0.5/1 mM). In some experiments, various tested antioxidant concentrations were added to the samples before ferrous iron and ascorbate (tocopherol, Trolox, rutine, PBN, desferrioxamine, EDTA). The final volume of the assay was 0.5 mL and the incubation was conducted at 37°C for 1 hour with concomitant flushing of pure oxygen at the top of the incubated membranes. Oxygen concentration was measured using an Oxymeter (OXI 530, WTW) on pooled membranes after incubation. The mean oxygen concentration (±SEM) was reproductively 760 ± 30 µM. After incubation, the initial volume of membrane was restored by Tris-HCl buffer and an aliquot taken for the malonaldehyde assay.

Lipid peroxidation measurement

The malonaldehyde formed during incubation was determined by HPLC, as described by Tomita and Okuyama [3]. A 0.2 mL volume of peroxidized membranes was reacted for 1 hr at room temperature with 0.2 mL of 2,4-dinitrophenylhydrazine (DNPH, 2.5 mg/mL in 1 M HCl) as derivatizing reagent. After centrifugation (5000 g for 10 min), 20 µL of the supernatant was injected into the HPLC apparatus. HPLC separations were performed on a Hypersil C18, 5 µ packed column (150 × 4.6 mm) with a mobile phase consisting of acetonitrile-0.01 M HCl (45:55, v/v) at a flow rate of 1.5 mL/min. Detection was realized by a UV spectrophotometric detector set at 310 nm (Spectra 100, Spectra Physics).

Binding assays

Binding assays were performed on preincubated homogenates after 3 washes in 50 mM Tris-HCl buffer pH 7.4 of the membranes to eliminate the various agents introduced during the first step.

[^{3}H]-flunitrazepam binding was measured on P_2 cortical membranes for CBR assay. The final volume of the binding assay was 500 µL and contained 300 µL membranes (0.10–0.15 mg protein) and 100 µL [^{3}H]-flunitrazepam (final concentration 7 nM) without (total binding) or with (nonspecific binding) 100 µL of clonazepam (final concentration 3 µM). Binding incubation was conducted at 4°C for 30 min and the reaction ended by rapid filtration through Whatman GF/B filters and 3 washes with 5 mL of 50 mM Tris-HCl buffer pH 7.4 using a 48-sample harvester (Brandel).

[^{3}H]-Ro 5-4864 binding was assayed as described by Marangos *et al.* [4] on liver homogenates for PBR assay. The final volume of the binding assay was 300 µL and contained 100 µL [^{3}H]Ro 5-4864 (final concentration 10 nM) and 200 µL membranes (0.2–0.3 mg protein). These membrane preparations were incubated with 5 µM Ro 5-4864 (nonspecific binding) or Tris-HCl buffer (total binding). Incubation was conducted at 4°C for 90 min and the reaction ended as described above.

In all binding experiments, filters were dried after the washing step and placed into scintillation vials with 10 mL Picofluor 15 (Packard) as scintillant. The radioactivity retained by filters was measured in a liquid scintillation spectrometer (Packard 1900 TR).

Influence of antioxidants on lipid peroxidation and binding of liver membranes. In the second part of the study various "antioxidants" were tested in the membrane model consisting of liver membranes incubated for 1 hour in a flux of oxygen at 37°C in presence of 0.5 mM ascorbate and 0.01 mM ferrous sulphate. The choice of this model was conditioned by the fact that these incubating conditions were the most peroxidative and that liver membranes were determined, in the first part of our study, as the most susceptible to binding loss consecutive to peroxidation.

The antioxidants tested were:

- Alpha-tocopherol (5/2/0.5 mM) and a water soluble analogue of vitamin E, Trolox C (0.1/0.05/0.01 mM);
- Rutin, a natural flavonoid (0.1/0.05/0.025/0.01 mM) and PBN (10/5/2 mM);
- Two chelating agents, desferrioxamine (20/10/2 mM) and EDTA (20/10/2 mM).

Malonaldehyde assays and [^{3}H]-Ro 5-4864 binding were realized as described above.

RESULTS

Influence of ferrous iron and ascorbate conditions on lipid peroxidation

Cerebral membranes. The effects of ascorbate and iron on lipid peroxidation are shown in Fig. 1A. There is a general trend towards increased peroxidation with ascorbate and Fe^{2+} concentrations. More precisely, this tendency was variable according to their relative concentrations:

- When Fe^{2+} was not added, MDA levels were slightly increased and dose-related to ascorbate;
- With an intermediary Fe^{2+} concentration (0.01 and 0.1 mM), MDA levels were significantly increased in presence of ascorbate. This effect is dose-related to ascorbate up to 0.5 mM. 1 mM ascorbate induced no more peroxidation;
- With high Fe^{2+} concentration (1 mM) high levels of MDA were obtained and were independent of ascorbate concentration.

Concerning the influence of ascorbate and iron on [^{3}H]-flunitrazepam binding it is clear from Fig. 1B that loss of binding was very limited. The most important decrease (22%–25%) occurred for 0.01 mM iron and 0.5/1 mM ascorbate. We shall see later that this relative "resistance" is characteristic of the cerebral CBR compared to the liver PBR.

Liver membranes. The effects of ascorbate and Fe^{2+} on lipid peroxidation of liver membranes are reported in Figure 2A. As observed with cerebral membranes, ascor-

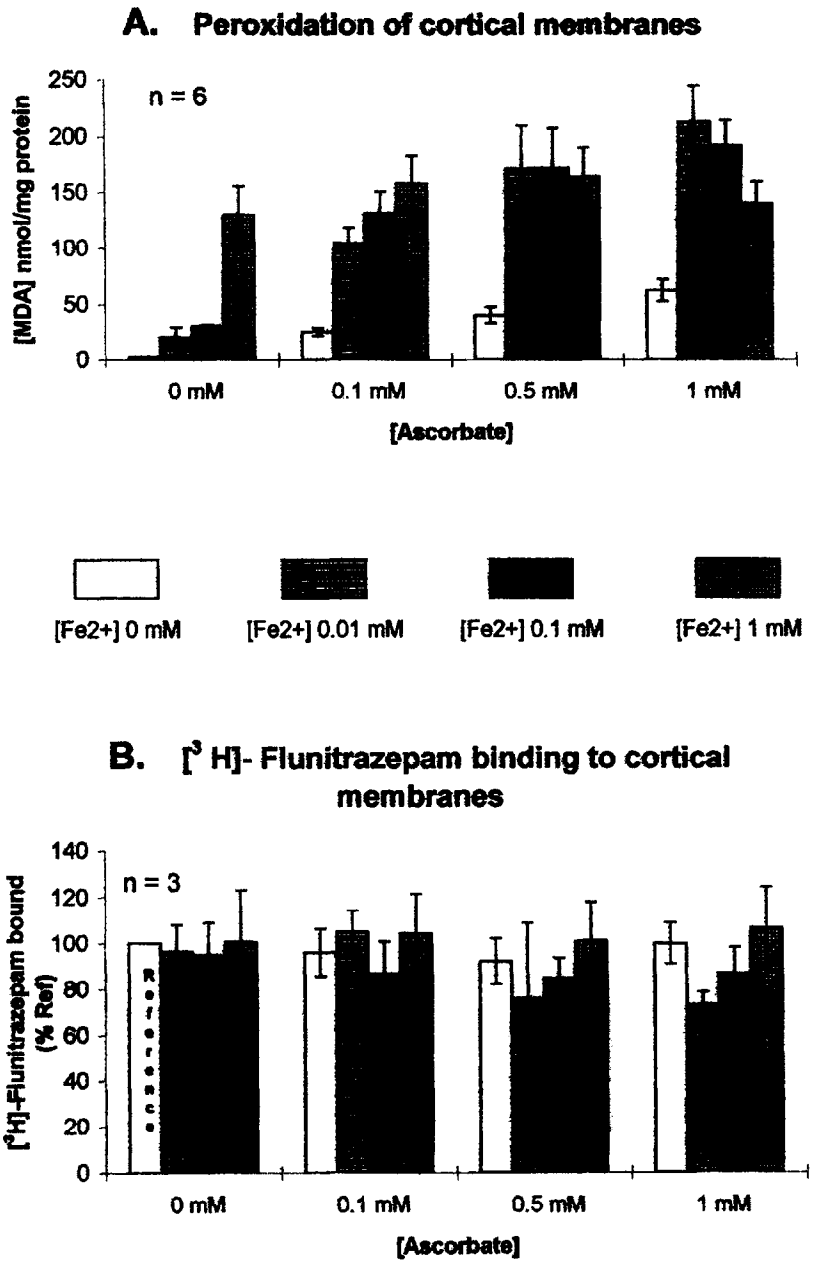

Fig. 1. Effects of oxidative conditions on peroxidation and [^{3}H]-flunitrazepam binding in cerebral membranes exposed *in vitro* to 760 µM oxygen. (A) Malonaldehyde levels obtained in cortical membranes incubated for 1 hr at 37°C in presence of various amounts of iron and/or ascorbate; (B) Residual [^{3}H]-flunitrazepam binding in cortical membranes as percent of reference binding. Reference was obtained on membranes incubated under 760 µM oxygen with no iron or ascorbate added. Data represented are means ± SEM of *n* experiments performed in duplicate (*n* values are indicated on the upper left).

bate and Fe^{2+} globally increased MDA production. However, this effect was also variable according to their relative concentrations:

- For assays without added Fe^{2+}, MDA levels were moderately increased in a dose-dependent manner with ascorbate (from 0.1 to 1 mM);
- For assays with low Fe^{2+} levels (0.01 mM), MDA concentrations were maximally increased and dose-related to ascorbate (from 0 to 1 mM);
- For 0.1 mM [Fe^{2+}], peroxidation was very limited (maximum for 0.1 mM ascorbate) and for 1 mM [Fe^{2+}] MDA levels were enhanced but were poorly related to ascorbate concentration.

Finally, the most peroxidative conditions appeared for 0.01 mM ferrous iron and 0.5/1 mM ascorbate.

Concerning peroxidative influences on [^{3}H]-Ro 5-4864 binding (Fig. 2B), losses of binding were in good agreement with malonaldehyde levels. The most important loss of binding occurred under higher peroxidative conditions (0/0.01 mM iron and 0.5/1 mM ascorbate), reaching 75%. In comparison, malonaldehyde levels in

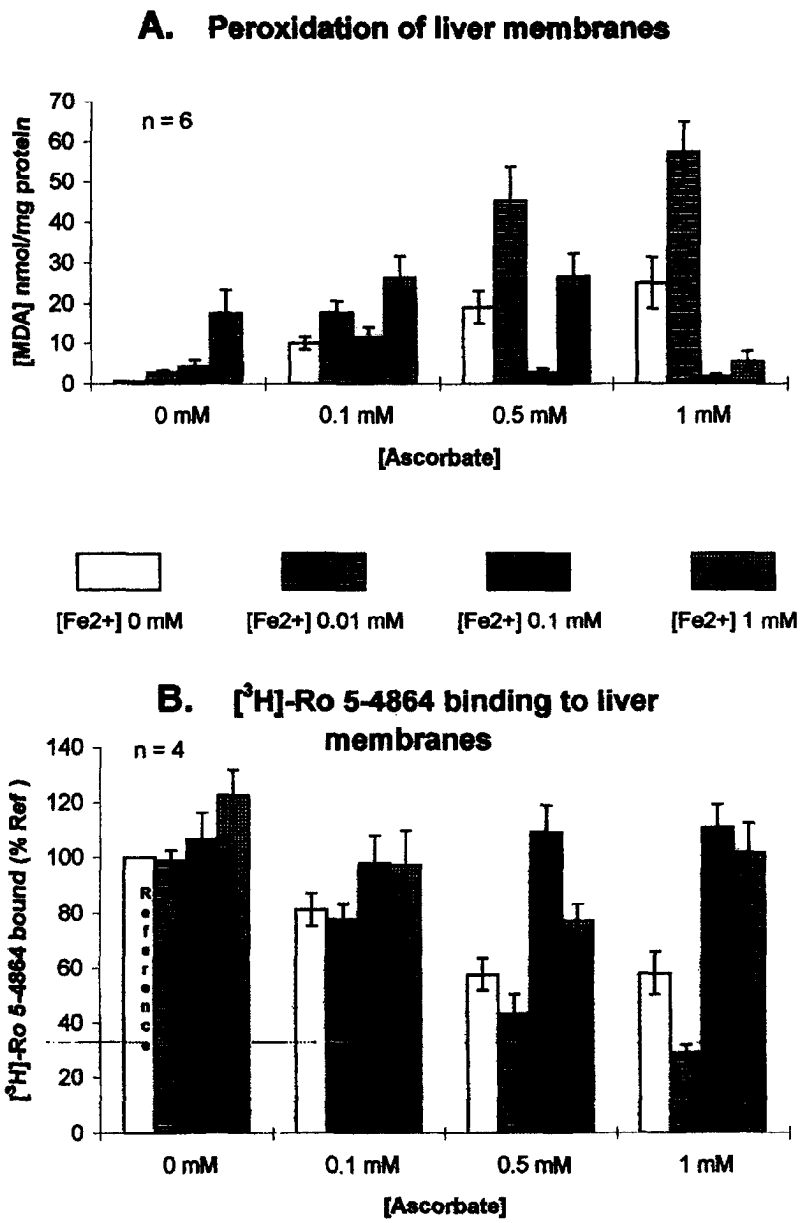

Fig. 2. Effects of oxidative conditions on peroxidation (malonaldehyde) and [^{3}H]-Ro 5-4864 binding in liver membranes exposed *in vitro* to 760 μM oxygen. (A) Malonaldehyde levels obtained in liver membranes incubated for 1 hr at 37°C in presence of various amounts of iron and/or ascorbate; (B) Residual [^{3}H]-Ro 5-4864 binding in liver membranes as percent of reference binding. Reference was obtained on membranes incubated under 760 μM oxygen with no iron or ascorbate added. Data represented are means ± SEM of *n* experiments performed in duplicate (*n* values are indicated on the upper left).

liver membranes were 4 to 6 times less extended than in cerebral membranes, even though binding losses were higher.

To explore the relationship between specific binding and peroxidation level, we performed a regression analysis of our data. Figure 3A reveals that [^{3}H]-Ro 5-4864 binding to liver membranes may be linearly and inversely correlated to the amount of MDA produced ($r = -0.67$, $P < 10^{-4}$). On the other hand, [^{3}H]-flunitrazepam binding to cerebral membranes did not appear to be correlated to MDA level, although the correlation coefficient was statistically significant ($r = -0.32$, $P < 0.03$, Fig. 3B).

These data strongly suggest that cerebral CBRs are less sensitive to membrane lipid peroxidation than liver PBRs and/or that the integrity of the lipid microenvironment is more essential for the binding capacity of PBR than for the binding capacity of CBR. This conclusion may only be drawn for the binding capacity of the receptor for a determined ligand and may not be extended to more general aspects, keeping in mind that our results only partially reflect receptor functioning.

Influence of antioxidants on lipid peroxidation and binding

Ascorbate and ferrous iron concentrations were 0.5 and 0.01 mM, respectively. Assays were conducted on crude liver homogenates for their higher susceptibility

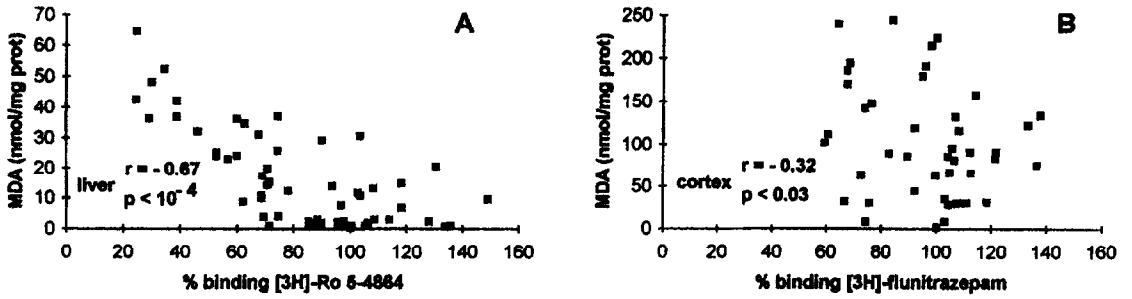

Fig. 3. Relationship between lipid peroxidation and specific drug binding. Regression analysis of data obtained in membrane samples incubated in various peroxidative conditions (under 760 μM oxygen). (A) Liver membranes and [^{3}H]-Ro 5-4864 binding; (B) Cerebral membranes and [^{3}H]-flunitrazepam binding.

than cerebral membranes in terms of binding loss. Peroxidative conditions were the same as described above, except that the tested antioxidant was introduced just before peroxidative incubation.

Tocopherol and trolox (Fig. 4A). Alpha-tocopherol is a well-known natural antioxidant. Under our testing conditions, it was introduced at a theoretical concentration of 0.5 to 5 mM; in fact, the real concentration of tocopherol was probably below these values because of the water-insoluble character of this antioxidant. At a higher tocopherol concentration (5 mM), lipid peroxidation was only slightly reduced and binding capacity was partially re-established whereas 0.5 and 2 mM tocopherol were not protective. In fact, it would be interesting to determine the real concentration of tocopherol in the aqueous/lipid phases to evaluate their respective contribution to peroxidation prevention. On the other hand, trolox, a water-soluble tocopherol derivative, seemed to be a good protector from 0.1 mM, since the binding capacity of liver membranes was restored and lipid peroxidation almost totally inhibited under these conditions.

Rutin and PBN (Fig. 4B). Rutin, a natural flavonoid, was chosen for its well-established properties against lipid peroxidation [5, 6] and its ability to scavenge superoxide radicals. In our model, rutin completely abolished peroxidation in a dose-dependent manner and was effective at concentrations as low as 0.05 mM, maintaining the initial binding capacity of liver membranes for Ro 5-4864.

PBN, selected as spin-trapping agent and effective in a model of ischemia [7], was not a good protective agent in our model because 10 mM were necessary for it to be effective as an antiperoxidant and binding-capacity protector.

Desferrioxamine and EDTA (Fig. 4C). These two agents were tested for the influence of their metal-chelating properties on the lipid peroxidation process. Chelating Fe^{3+} may prevent its contribution to reactions implicated in lipid peroxidation. In our model, 0.02 mM desferrioxamine was sufficient to completely abolish lipid peroxidation. 0.02 mM EDTA was also efficient although to a slightly lesser extent. Because no protection was obtained at 0.01 mM of these two chelating agents, and 0.01 mM Fe^{2+} was initially introduced, we concluded that concentrations of chelators in excess of Fe^{2+} were required for potent protection.

DISCUSSION

Peroxidative conditions and binding consequences

The primary purpose of this study was to evaluate the relative importance of the contribution of lipid peroxidation on the loss of the binding of membrane receptors for a specific ligand.

Concerning the influence of ascorbate on lipid peroxidation, it is well-known that its properoxidant influence is mediated through an iron-dependent peroxidative mechanism [8]. Iron is widely distributed in enzymes and hemoproteins and probably constitutes a key component in peroxidative damages; it has been proposed that the Fe^{3+} to Fe^{2+} ratio is more essential to initiate peroxidation than the absolute concentration of Fe^{3+} and Fe^{2+} [9–11]. In our model, we only tested the initial conditions of the peroxidative incubation consisting of the Fe^{2+} introduced. It seems likely that oxygen oxidizes both ferrous iron and ascorbate during incubation and that the ferric to ferrous ratio evolves relative to the initial condition. On the other hand, under the same conditions, cortical membranes lead to the upper values of malonaldehyde, unlike liver membranes. This phenomenon is probably due to the relative richness of cerebral membranes in polyunsaturated fatty acids. This indicates that the susceptibility to lipid peroxidation of membranes from diverse origins may be related directly to their lipid composition, but that the consequences of such peroxidative processes on binding capacity are not necessarily related to the degree of peroxidation. The decrease in binding capacity depends on the relative contribution of the lipid environment to the binding process or on the direct susceptibility of the glycoproteic moiety of the receptor to oxidative damage.

Influence of antioxidants

Under our conditions, rutin, trolox, and metal chelators (desferrioxamine and EDTA) are good inhibitors of lipid peroxidation and are consequently good protectors of drug binding. Trolox is essentially a radical scavenger [12], desferrioxamine and EDTA are metal chelators and rutin possesses both properties [5]. These various possibilities of protection do not permit us to conclude in favor of a unique mechanism of peroxidation. It could also be said that a unique mechanism of peroxidation may be inhibited by diverse antioxidants intervening at different stages of the peroxidative process.

Many experiments on the influences of peroxidative or oxidative conditions on various membrane receptors have previously been reported. Dopamine [8, 13, 14], alpha-adrenergic [13], beta-adrenergic [15], muscarinic [16], serotonin [14], adenosine [17], NMDA [18], and CBR and PBR [19] from diverse membrane origins have been studied. All these experiments have led their authors to conclude that there is a loss of binding consecutive to peroxidation induced *in vitro* by ascorbate, Fe^{2+}/ascorbate, or phospholipase A_2. The global interest of

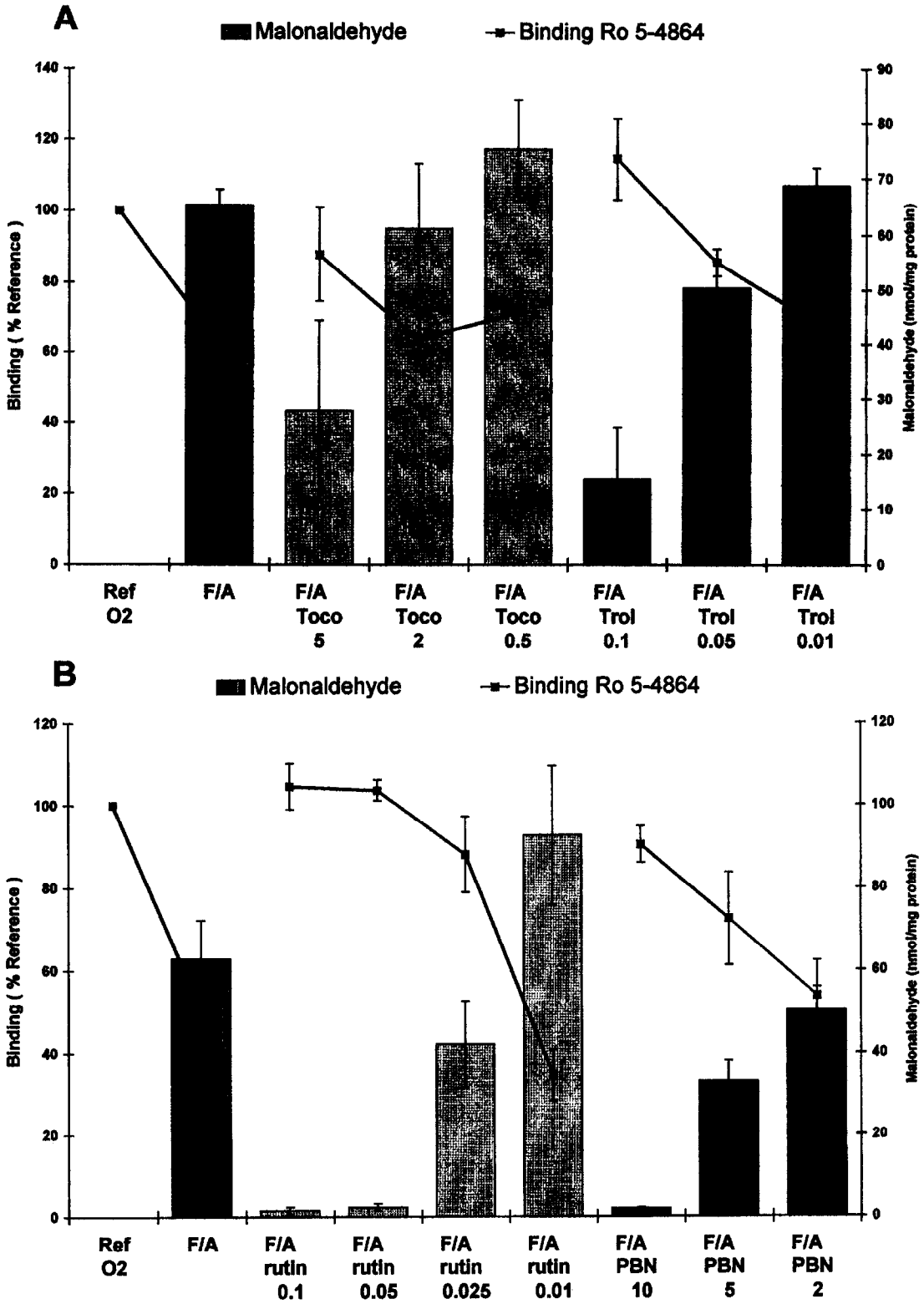

Fig. 4. Protective effects of antioxidants on liver membranes incubated for 1 hr at 37°C in presence of 0.01 mM $FeSO_4$ and 0.5 mM ascorbate (under 760 μM O_2). The degree of protection is evaluated by the ability to inhibit malonaldehyde production (represented as graphic bars) and the ability to maintain [^{3}H]-Ro 5-4864 binding (represented as plain squares). Data presented are means ± SEM of 4 to 6 independent experiments performed in duplicate. (A) Antioxidant capacity of tocopherol and trolox; (B) Antioxidant capacity of rutin and PBN; (C) Antioxidant capacity of desferrioxamine and EDTA. Abbreviations used on abscissa: Ref O_2 = reference under oxygen (760 μM O_2) in the absence of ferrous iron and ascorbate; F/A = 760 μM Oxygen plus ferrous iron/ascorbate (0.01 and 0.5 mM, respectively); Other data with antioxidants = 760 μM oxygen plus ferrous iron/ascorbate plus antioxidants (values indicate their initial concentration expressed in mM); Toco = tocopherol; Trol = trolox; PBN = phenylbutylnitrone; Desfer = desferrioxamine; EDTA = ethylene diamine tetra acetate.

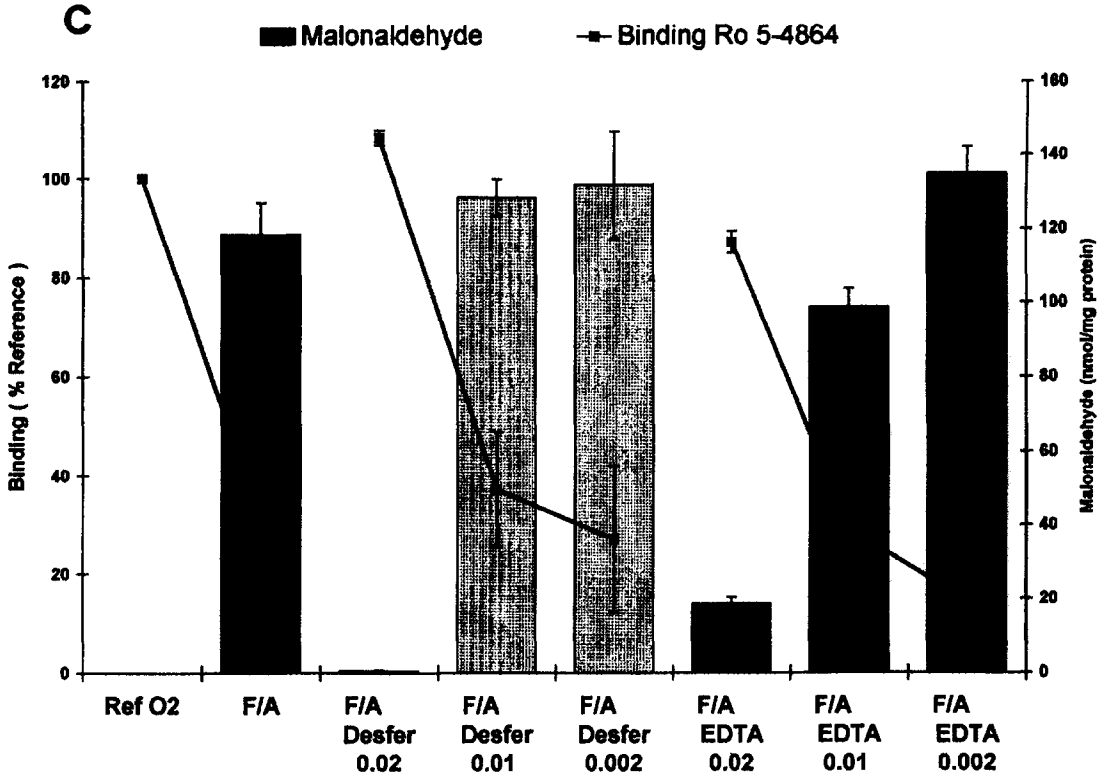

Fig. 4. Continued.

such results is attenuated by the fact that these data were obtained under a great variety of oxidative conditions. It seems obvious that receptor binding is classically altered by peroxidation, but it seems important to have access to the relative sensitivity of numerous receptors under the same oxidative conditions. This "*in vitro*" approach might constitute a first step in our knowledge of membrane receptor susceptibility to oxidative damage and could ultimately be completed by additive studies taking into account the natural environment of receptors in physiological and pathological conditions.

In a previous study, we investigated the consequences of hyperbaric oxygen-exposed rats (O_2 350 kPa, 2 hr) on binding capacities of liver and cerebral membranes [1]. We described a diminished binding capacity of cerebral membranes (30%) for flunitrazepam (corresponding to CBRs) after hyperbaric oxygen exposure, whereas no such alteration was obtained concerning the binding capacity of liver and cerebral membranes for Ro 5-4864 (corresponding to PBRs). This could be interpreted as demonstrating a better "resistance" of PBRs than CBRs. In fact, the present study seems to show the opposite because under the same *in vitro* oxidative conditions, PBRs are more affected than CBRs. A possible explanation may be that in a natural environment the mitochondrial outer membrane, where PBRs are preferentially located, is constitutively "protected" by lipids and proteins normally adequate for weak pO_2 values. It seems likely that our *in vitro* model provides a very aggressive hyperoxic environment for mitochondrial membranes, which are known to be particularly rich in polyunsaturated fatty acids. On the other hand, synaptosomal membranes are probably more protected against hyperoxic challenge because of their relatively common high pO_2 values.

We conclude that *in vitro* studies on the influence of "oxidative stress" on membrane receptors are important for a better understanding of receptor functioning, but that conclusions about biochemical mechanisms have to be grounded in realistic aspects of the natural environment, including cellular compartmentation. As previously described [20, 21], *in vitro* studies conducted to test putative antioxidants are not sufficient and additional experiments performed *in vivo* are required.

REFERENCES

1. Courtière A, Reybaud J, Camilla C, Lobert P, Drouet J and Jadot G, Oxygen-induced modifications of benzodiazepine receptors and D2 dopamine receptors in the rat under hyperoxia. *Free Rad Res Commun* **15:** 29–34, 1991.
2. Squires RF and Braestrup C, Benzodiazepine receptors in rat brain. *Nature* **266:** 732–734, 1977.
3. Tomita M and Okuyama T, Determination of malonaldehyde in oxidized biological materials by high-performance liquid chromatography, *J Chromatogr* **515:** 391–397, 1990.
4. Marangos PJ, Patel J, Boulenger JP and Clark-Rosenberg R, Characterization of peripheral-type benzodiazepine binding sites in brain using [^{3}H]-Ro 5-4864. *Mol Pharmacol* **22:** 26–32, 1982.
5. Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA and Potapovitch AI, Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem Pharmacol* **38:** 1763–1769, 1989.
6. Nègre-Salvayre A, Reaud V, Hariton C and Salvayre R, Protective effect of α-tocopherol, ascorbic acid and rutin against peroxidative stress induced by oxidized lipoproteins on lymphoid cell lines. *Biochem Pharmacol* **42:** 450–453, 1991.
7. Carney J and Floyd RA, Protection against oxidative dam-

age to CNS by α-phenyl-tert-butyl nitrone (PBN) and other spin-trapping agents: A novel series of nonlipid free radical scavengers. *J Mol Neurosci* **3:** 47–57, 1991.

8. Heikkila RE, Cabbat FS and Manzino L, Inhibitory effects of ascorbic acid on the binding of [^{3}H]dopamine antagonists to neostriatal membrane preparations: relationship to lipid peroxidation. *J Neurochem* **38:** 1000–1006, 1982.
9. Braughler JM, Duncan LA and Chase RL, The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation. *J Biol Chem* **261:** 10282–10289, 1986.
10. Beach DC and Giroux E, Inhibition of lipid peroxidation promoted by iron (III) and ascorbate. *Arch Biochem Biophys* **297:** 258–264, 1992.
11. Minotti G and Aust SD, Redox cycling of iron and lipid peroxidation. *Lipids* **27:** 219–226, 1992.
12. Britt SG, Chiu WS, Redpath GT and VandenBerg SR, Elimination of ascorbic acid-induced membrane lipid peroxidation and serotonin receptor loss by Trolox-C, a water soluble analogue of vitamin E. *J Receptor Res* **12:** 181–200, 1992.
13. Leslie FM, Dunlap CE III and Cox BM, Ascorbate decreases ligand binding to neurotransmitter receptors. *J Neurochem* **34:** 219–221, 1980.
14. Muakkassah-Kelly SF, Andresen JW, Shih JC and Hochstein P, Decreased [^{3}H]serotonin and [^{3}H]spiperone binding consequent to lipid peroxidation in rat cortical membranes. *Biochem Biophys Res Commun* **104:** 1003–1010, 1982.
15. Heydeck D, Haberland A, Schimke I and Will-Shahab L, The effect of Fe^{2+}/ascorbate induced lipid peroxidation on the beta-adrenoceptor of sarcolemmal membranes. *Agents Actions* **32:** 146–148, 1991.
16. Ghosh C, Dick RM and Ali SF, Iron/ascorbate-induced lipid peroxidation changes membrane fluidity and muscarinic cholinergic receptor binding in rat frontal cortex. *Neurochem Int* **23:** 479–484, 1993.
17. Fonseca E and Ribeiro JA, Ascorbate-induced lipid peroxidation and inhibition of [^{3}H]adenosine binding to rat brain synaptosomes. *Nucleosides Nucleotides* **10:** 1213–1214, 1991.
18. Goel R, Mishra OP, Razdan B and Delivoria-Papadopoulos M, Modification of NMDA receptor by in vitro lipid peroxidation in fetal guinea pig brain. *Neurosci Lett* **151:** 219–223, 1993.
19. Havoundjian H, Cohen RM, Paul SM and Skolnick P, Differential sensitivity of "central" and "peripheral" type benzodiazepine receptors to phospholipase A_2. *J Neurochem* **46:** 804–811, 1986.
20. Halliwell B, How to characterize a biological antioxidant. *Free Rad Res Commun* **9:** 1–32, 1990.
21. Lin Y and Jamieson D, Effects of antioxidants on oxygen toxicity in vivo and lipid peroxidation in vitro. *Pharmacol Toxicol* **70:** 271–277, 1992.