Influence of lipid peroxidation on β-adrenoceptors

K. Kramer, B. Rademaker, W.H.M. Rozendal, H. Timmerman and A. Bast

Dept of Pharmacochemistry, Subfaculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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The peroxidation of lipids in biological membranes is a destructive phenomenon that can be elicited in various ways. Surface receptor molecules that allow cells to respond to hormones are possibly inactivated during lipid peroxidation. Effects of lipid peroxidation on receptors have not been extensively examined thus far. This investigation shows that there is a decrease in β-adrenoceptor density (measured as specific (−)-[^{125}I]iodocyanopindolol binding) during lipid peroxidation, in both lungs and erythrocytes of the rat. To this end, lung membranes (containing both β_1- and β_2-adrenoceptors) and intact erythrocytes (containing a homogeneous β_2-adrenoceptor population) were pretreated with cumene hydroperoxide (lung membranes with 0.1 mM and erythrocytes with 1 mM) and Fe^{2+} (1×10^{-5} M) for 60 min which resulted in extensive lipid peroxidation measured as malondialdehyde formation. The ratio β_1-:β_2-adrenoceptor density in lung membranes after treatment with cumene hydroperoxide did not change and remained at 30%:70%. A single injection (i.p.) with the herbicide paraquat (50 mg/kg, 24 h), which is known to cause lung damage via lipid peroxidation, resulted in similar alterations in receptor density to those caused by cumene hydroperoxide in the in vitro experiments.

Lipid peroxidation *β-Adrenoceptor* *(Lung)* *Erythrocyte* *Receptor binding*

1. INTRODUCTION

Lipid peroxidation has been defined as the 'oxidative deterioration of polyunsaturated lipids', i.e. lipids that contain more than two carbon-carbon double bonds [1]. Because the membranes surrounding cells and cell organelles contain large amounts of polyunsaturated fatty acid side chains, most of the interest in this subject in the literature has been focussed on polyunsaturated fatty acids. Lipid peroxidation of polyunsaturated fatty acids leads to the formation of malondialdehyde, a product known to be toxic to a variety of biological systems [2–5]. Malondialdehyde is formed directly from peroxy (free) radicals and interacts with a variety of cellular constituents to form polymers that are believed to play a role in formation of age-related lipopigments [5]. Oxygen radicals have been suggested as a major cause of some heart and lung diseases and aging. Oxygen radicals and other oxidants appear to be toxic in large part because they initiate the chain reaction of lipid peroxidation. This process changes all kinds of enzymatic reactions. We investigated the effect of lipid peroxidation on β-adrenoceptors of lungs and erythrocytes of the rat, both in vitro and in vivo. To this end we pretreated lung membranes and intact erythrocytes in vitro with cumene hydroperoxide and Fe^{2+}, which resulted in extensive lipid peroxidation. In the in vivo situation we administered a single injection (i.p.) of the herbicide paraquat, which is known to cause lung damage via lipid peroxidation [6,7], and studied β-adrenoceptor density after 24 h. The effect of lipid peroxidation on β-adrenoceptors of lungs (β_1 and β_2) and erythrocytes (β_2) of the rat was measured with a receptor-binding assay using (−)-[^{125}I]iodocyanopindolol [8].

2. MATERIALS AND METHODS

2.1. *Preparation and incubation of erythrocytes*

After decapitation of male Wistar rats of 180–200 g, the blood was collected in heparin

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tubes (Greiner, Alphen a/d Rijn, The Netherlands) and centrifuged (3000 rpm, 3 min). The supernatant was removed and 6 ml buffer A, containing 140 mM NaCl, 50 mM Tris and 5 mM $MgCl_2$ (pH 7.4 at 0°C), was added. This was repeated 3 times at 4°C. The final pellet of blood was suspended in buffer A (1:16, pH 7.4 at 37°C) and pretreated with 1 mM cumene hydroperoxide and Fe^{2+} (1×10^{-5} M) for 60 min at 37°C. After this incubation, the erythrocytes were lysed with 10 mM phosphate buffer (pH 7.4) for 5 min at 4°C and centrifuged at 50000 × *g* for 30 min at 4°C. This procedure was repeated 3 times. The receptor-binding assay was performed with membranes (4–10 μg protein/ml) suspended in buffer A (pH 7.4 at 37°C).

2.2. *Preparation and incubation of lung membranes*

After decapitation of male Wistar rats of 180–200 g, the lungs were removed and homogenized in buffer A (1 g lung in 3 ml buffer A, pH 7.4 at 37°C) with a Polytron (PT 10/35) homogenizer (1 × 15 s, max. speed). This crude preparation was centrifuged for 20 min at 10000 × *g*. The supernatant was centrifuged for 60 min at 50000 × *g*. The pellet obtained was washed in buffer A (pH 7.4 at 0°C) and homogenized (1 min, max. speed). The whole procedure was performed at 4°C. This final pellet was taken up in buffer A (pH 7.4 at 37°C, 1:15) and pretreated with 0.1 mM cumene hydroperoxide and Fe^{2+} (1×10^{-5} M) for 60 min at 37°C. After the incubation, buffer A (pH 7.4 at 0°C) was added (1:10) and centrifugation at 50000 × *g* for 30 min followed. After homogenization of the pellet (1 min, max. speed) a second centrifugation was carried out. The receptor-binding assay was performed using membranes (15–21 μg protein/ml) suspended in buffer A (pH 7.4 at 37°C).

2.3. *Pretreatment with paraquat*

A group of male Wistar rats (180–200 g) were pretreated with a single injection (i.p.) of 50 mg/kg with the herbicide paraquat. The control group was pretreated with a physiological salt solution [6]. After 24 h the rats were decapitated, the blood collected and the lungs removed. Preparation of erythrocyte membranes and of the lungs was performed as described above.

2.4. *Determination of malondialdehyde and protein*

Lipid peroxidation was assayed by measuring thiobarbituric acid (TBA)-reactive material. An aliquot of the incubation mixture (0.3 ml) was stopped by mixing with ice-cold TBA-trichloroacetic acid-HCl-butylhydroxytoluene (BHT) solution (2 ml). After heating (15 min, 80°C) and centrifugation (15 min) the absorbance at 535 nm vs 600 nm was determined. The TBA-trichloroacetic acid-HCl solution was prepared by dissolving 41.6 mg TBA/10 ml trichloroacetic acid (16.8%, w/v in 0.25 N HCl). To 10 ml TBA-trichloroacetic acid-HCl 1 ml BHT (1.5 mg/ml ethanol) was added. The added chemicals did not interfere with the assay at the concentrations used. Protein was assayed as in [9], using bovine serum albumin as a standard.

2.5. *Receptor-binding assay*

All assays were performed in triplicate in a final volume of 350 μl. For the saturation experiments the lung membranes and erythrocytes were incubated for 60 min at 37°C with (−)-[^{125}I]iodocyanopindolol (0–200 pmol/l). In all experiments non-specific binding was determined with 10^{-6} M timolol. At the end bound and free ligands were separated by dilution of the sample with 4 ml cold buffer A (pH 7.4 at 0°C), followed by rapid filtration through Whatman G/FC filters on a Millipore vacuum filtrator. Each filter was washed twice with 4 ml cold buffer A and counted for radioactivity using an Auto-Gamma scintillation spectrometer (Packard, USA).

2.6. *Analysis of data*

B_{max} values (fmol/mg protein) and dissociation constants, K_d (pM), were evaluated from the binding data, according to the model based on the first-order mass action law for multiple binding sites. This was done with the non-linear curve-fitting program (LIGAND) [10] on a Zenith Z-110 microcomputer. This program provides a correction for real free ligand concentrations on non-specific binding and a statistically reliable analysis of binding data. Fits for multiple binding sites were considered significantly best when the *P* value for a single binding site was less than 0.05.

2.7. *Chemicals*

Drugs used were: (−)-timolol (Merck, Sharp and Dohme); ICI 89,406 (ICI); cumene hydroperoxide (ICN, Rare and Fine Chemicals); paraquat (Sigma); $FeSO_4$ (Merck); (−)-[^{125}I]iodocyanopindolol (spec. act. 1800 Ci/mmol) (New England Nuclear). All other reagents used were of reagent grade.

3. RESULTS

Fig.1 shows that incubation of erythrocytes or lung membranes with cumene hydroperoxide and Fe^{2+} results in time-dependent formation of TBA-reactive material, which is mainly malondialdehyde. Fig.2 shows the changes in the saturation of iodocyanopindolol binding to the homogeneous population of β_2-adrenoceptors on the membranes of erythrocytes, due to pretreatment of intact erythrocytes with cumene hydroperoxide (1 mM) and Fe^{2+} (1×10^{-5} M). Analysis of the binding data resulted in a K_d of 26 ± 4 pM and a B_{max} of 947 ± 72 fmol/mg protein for membranes of control erythrocytes and a K_d of 42 ± 10 pM and a B_{max} of 307 ± 42 fmol/mg protein for membranes of cumene hydroperoxide of pretreated erythrocytes. Pretreatment of lung membranes with cumene hydroperoxide (0.1 mM) and Fe^{2+} (1×10^{-5} M) for 60 min also produced

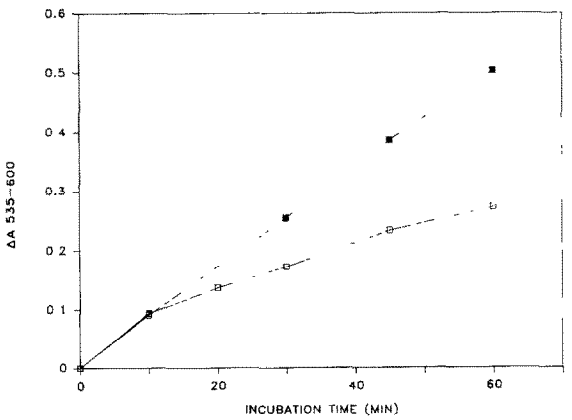

Fig.1. Formation of TBA-reactive material during incubation with cumene hydroperoxide and Fe^{2+}. (□) Erythrocytes (61–64 μg protein/ml) with 1 mM cumene hydroperoxide and 1×10^{-5} M Fe^{2+}. (■) Lung membranes (100–107 μg protein/ml) with 0.1 mM cumene hydroperoxide and 1×10^{-5} M Fe^{2+}.

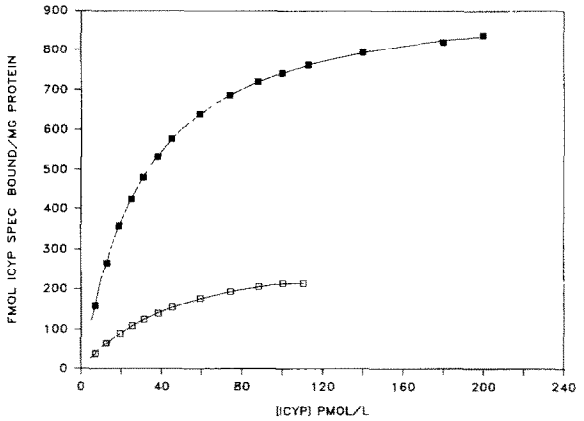

Fig.2. Specific binding of increasing concentrations of iodocyanopindolol (ICYP) in erythrocyte membranes. Control erythrocytes (■) or membranes of erythrocytes pretreated with 1 mM cumene hydroperoxide and Fe^{2+} (1×10^{-5} M) (□). Incubation took place with ^{125}ICYP for 60 min at 37°C. Non-specific binding was determined by addition of 10^{-6} M timolol. Control (■), K_d = 26 ± 4 pM and B_{max} = 947 ± 72 fmol/mg protein; with cumene hydroperoxide (□), K_d = 42 ± 10 pM and B_{max} = 307 ± 47 fmol/mg protein. The results were obtained by a simultaneous fit of two experiments.

changes in both β-adrenoceptor density (B_{max}) and dissociation constant (K_d) (table 1).

It is known that lung membranes contain both β_1- and β_2-adrenoceptors. Here we used the selective β_1-adrenergic ligand ICI 89,406 to quantify the β_1- and β_2-receptor populations (fig.3). The ratio β_1-:β_2-adrenoceptor density in lung membranes after in vitro treatment with cumene hydroperoxide did not change and remained at 30%:70%.

Table 1

Binding characteristics of ICYP to lung membranes of the rat with or without pretreatment with cumene hydroperoxide

Pretreatment	K_d (pM)	B_{max} (fmol/mg protein)
–	112 ± 11	1250 ± 96
Cumene hydroperoxide	82 ± 5	881 ± 37

Results (± SE) were obtained by simultaneous fitting of two independent experiments

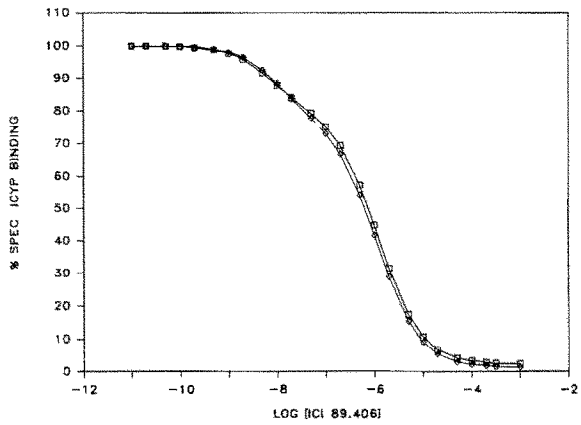

Fig.3. Determination of the ratio β_1-:β_2-adrenoceptor density by displacement of specific binding of ICYP (35 pM) by β_1-selective adrenergic ligand ICI 89,406 from either control (□) or cumene hydroperoxide (0.1 mM) and Fe^{2+} (1×10^{-5} M) (◇)-pretreated lung membranes. Curves were drawn after computer analysis. Ratio of β_1:β_2: (control) 28 ± 5%:72 ± 4% and (pretreated) 32 ± 6%:68 ± 4%.

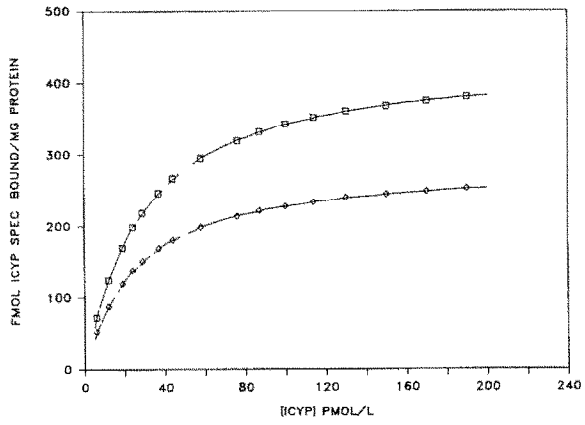

Fig.4. Specific binding of increasing concentrations of ICYP in erythrocyte membranes. Erythrocytes were obtained from either control rats (□) or rats pretreated with paraquat (◇) (50 mg/kg, 24 h). Incubation took place with ICYP for 60 min at 37°C. Non-specific binding was determined by the addition of 10^{-6} M timolol. Control (□), K_d = 24 ± 2 pM and B_{max} = 432 ± 14 fmol/mg protein; pretreated with paraquat (◇), K_d = 23 ± 4 pM and B_{max} = 284 ± 21 fmol/mg protein. A representative experiment is shown.

Lipid peroxidation initiated in vivo by a single paraquat administration (i.p., 50 mg/kg, 24 h) resulted in a decrease in specific (−)-[^{125}I]iodocyanopindolol binding in lung membranes as indicated in table 2. Also, membranes of erythrocytes possess less β-adrenoceptors after paraquat pretreatment, whereas the K_d value remained identical. Analysis of the binding data resulted in a B_{max} and a K_d of 432 ± 14 fmol/mg protein and 24 ± 2 pM in control and 284 ± 21 fmol/mg protein and 23 ± 4 pM, respectively, after paraquat pretreatment.

Table 2

Binding characteristics of ICYP to lung membranes of the rat with or without pretreatment with paraquat

Pretreatment	K_d (pM)	B_{max} (fmol/mg protein)
−	85 ± 16	1967 ± 371
Paraquat	53 ± 5	1561 ± 99

The result is from a representative experiment ± SE

4. DISCUSSION

The major constituents of biological membranes are lipids and proteins. It is well known that the process of lipid peroxidation can damage these membrane constituents. Here, we found a decrease in both affinity (K_d values) and number of β-adrenoceptors (B_{max} values) after lipid peroxidation both in vitro and in vivo. This can be explained by general deterioration of the membrane. Moreover, it is known that the β-adrenoceptors retain essential sulfhydryl groups [11]. Our results may therefore also be explained by means of the effect of aldehydes which are formed during lipid peroxidation and may interact with sulfhydryl moieties of β-adrenoceptors. β_1- and β_2-adrenoceptors seem to be equally sensitive to lipid peroxidation, since the ratio β_1-:β_2-adrenoceptors remained the same after lipid peroxidation in lung membranes.

Our results also provide an explanation for the observations by Engels et al. [12], who reported a decrease in tracheal relaxation in response to isoproterenol by oxygen radicals produced by macrophages.

Other types of receptors may also be affected by free radicals. It has been found [13] that specific binding of 5-[^{3}H]hydroxytryptamine to washed ascorbate-pretreated cortical membranes of the rat was significantly lower than their incubated controls. It has been recently reported [14] that pretreatment of the cerebral synaptic membranes with hydrogen peroxide resulted in a significant suppression of specific binding of [^{3}H]muscimol, which is a radioligand for the γ-aminobutyric acid receptor. Based on our results, an overall decrease in β-receptor-mediated response should be expected after lipid peroxidation. This may have important implications in pathophysiological conditions in which lipid peroxidation is thought to play an essential role. For example, lipid peroxidation has been associated with aging [15,16], and in fact the β-receptor response decreases with age [17]. Also, tissue damage induced by ischemia or hypoxia has been suggested to be due to oxidative stress [18]. Alterations in β-receptor response under these conditions have not been examined thus far. The toxicity of many compounds, like paraquat, has been shown to proceed via lipid peroxidation. Here we found alterations in both β-adrenoceptor density in lungs as well as in erythrocytes after administration of the herbicide paraquat. It is possible that the changes observed in erythrocytes merely form a reflection of the severe lung damage induced by the high dosage of paraquat. It has been suggested that the effect of oxidative stress in lungs is partly diminished by the protective action of blood cells [19]. Because blood cells are easily obtainable, also in the case of humans, it should be interesting to elucidate these secondary changes in erythrocytes further. This therefore constitutes part of our present investigations.

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